BLOOD TRANSFUSION

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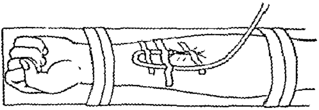
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BLOOD TRANSFUSION



Illustrated With Two Hundred Diagrammatic Drawings

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Preface

Since the first blood bank in the United States was established in 1937 there has been a great increase in the therapeutic use of blood and its derivatives and, consequently, a steady growth in the number of blood banks. Transfusion services have been developed by the trial-and-error method by their founders. Much of the hard-earned experience obtained in this manner has not been formally recorded, but has been passed on by word of mouth or in personal correspondence. This state of affairs was brought into sharp focus during World War II when the Federal Government encouraged the establishment of civilian blood banks as a measure for national defense. There was no single publication which dealt adequately with the problems of organizing and operating a blood transfusion service. Partially to fill this need *A Technical Manual on the Preservation and Transfusion of Blood* was published in November 1942 by the U. S. Office of Civilian Defense, with a companion pamphlet entitled *A Technical Manual on Citrated Human Blood Plasma*. The material in these, revised and extended, appeared in March 1944 under the title *The Operation of a Hospital Transfusion Service, OCD Publication 2220.* As two of us (E.L.D. and J.B.A.) collaborated with others in writing and revising this handbook, we were conscious of its many inadequacies, some of which were due to the necessity for brevity and quick publication. In the meantime, overseas, the third of the present authors (R.C.H.) was faced with the problem of teaching the facts of blood transfusion to army personnel. He also regretted the absence of a suitable text.

In the present work we have chosen to cover the entire field of blood transfusion rather than to limit its scope to a consideration of either whole blood or plasma. A major portion of the text has been devoted to the various aspects of whole blood transfusion because it is our considered judgment, as clinicians, that whole blood is still, and will continue to be, the major need in the treatment of patients. In addition, the management of whole blood transfusions is a more intricate subject than is the processing and administration of plasma or its derivatives.

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It has been our purpose to include material which is of interest to (1) physicians and medical students whose patients receive transfusions, (2) physicians and others who supervise transfusion services, and (3) laboratory technicians who perform the tests and procedures necessary in modern transfusion. For the convenience of the latter group, the step-by-step description of laboratory tests has been segregated in a separate section. Each step in some of the procedures has been illustrated in such a way that the margins of the pages constitute a series of flow diagrams. This arrangement permits the technician to follow the steps by means of the pictures, once he has become fairly familiar with the material.

All three authors are internists who have been interested in blood transfusion for ten years or more. We believe that a clinical background is desirable, if not imperative, for the understanding of some problems of a transfusion service. It is with some regret that we have watched the development of knowledge and technique to the point where it must now be acknowledged that blood transfusion is a minor medical specialty. The necessity for this is genuine; the regret is that blood transfusion cannot be carried out as simply as "in the good old days."

It might be argued that blood transfusion is merely an extension of the laboratory service and that there are competent laboratory workers who practice blood transfusion. In rebuttal, it should be noted that the physician-patient relationship involves the transfusionist. The worker who collects blood from a person assumes responsibility for the donor's welfare during a procedure which is sometimes hazardous. The diagnosis and treatment of the patient with a transfusion reaction is likewise within the clinician's province. The methods of blood transfusion can be taught to a person without medical training, just as surgical procedures can, but the final judgment and direction should rest with a physician who has specialized in the field.

Any writer on blood transfusion is faced with the problem of the selection of references to the medical literature. There are probably more than 10,000 papers on blood transfusion or some of its aspects, so that it is doubtful that any one person has read them all. To make a comprehensive survey of the entire literature would be impractical and unrewarding. We have preferred to cite reference to relatively few articles which we have read and consider important in the light of our experience. There has been no hesitation to indicate clearly our judgment and opinions on many subjects. Although the writer who adopts such a policy is assuming a considerable responsibility, it seems to us the better

alternative in rendering assistance to those of our readers who are relatively unfamiliar with the field.

We are keenly aware that no first edition of a book on blood transfusion can at present contain all the unrecorded worthwhile information which has been accumulated by the experience in many blood banks. Rarely have we visited a blood bank without encountering new problems and solutions to them with which we were previously unfamiliar. Therefore it has seemed to us that this book would be most helpful by describing our experiences in somewhat immodest detail in the hope that the reader will apply the results if they seem to help solve his own problems.

The authors desire especially to thank Mr. Dale Ballantyne, instructor in art at the State University of Iowa, for making the illustrations. He has shown a keen insight into the problem of visual presentation of a rather difficult subject.

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CHAPTER 1

Historical Perspective

By Elmer L. DeGowin

The detailed history of blood transfusion is a fascinating subject for physician or layman but the reader frequently finds difficulty in bringing many events into proper perspective. DeBakey[1] has written an excellent summary of the salient discoveries in the field up to 1915. It is rather the purpose of this discussion to attempt an evaluation of the various advances in the light of their effect in making transfusion available to the average patient.

THE FIRST 275 YEARS

The Period of Exploration. William Harvey first announced his observations on the circulation of the blood in his lectures in 1616, the year of Shakespeare's death. They were published in 1628 in his famous monograph.[2] It seems probable that Richard Lower, the English physiologist, performed the first successful transfusion on animals in 1665.[3] The first well-documented transfusion to man was probably performed by Jean Baptiste Denis in 1667,[4] who transfused the blood of a lamb into a youth. The operation was performed but rarely for the next 150 years. There was no consensus as to the indications, and many disappointments were encountered because of the fantastic results expected. About 1818 James Blundell, the English physiologist and obstetrician, revived the procedure and advocated its use in the treatment of acute hemorrhage.[5] He also insisted that human blood was more effective than that of animal origin. His writings probably introduced the subject to the American profession.

But in spite of centuries of discussion, the procedure was so impractical that Landois[6] in 1875 was able to collect reports of only 347 transfusions from the literature of the world up to that

● PART VI PREPARATION AND ADMINISTRATION OF PLASMA

● PART VII PREPARATION AND ADMINISTRATION OF THE BLOOD DERIVATIVES AND PLASMA SUBSTITUTES

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the operation was within the skill of anyone who could insert a needle into a vein.

The procedure immediately became popular and was employed quite extensively in the treatment of the wounded in World War I. Its use has steadily grown ever since in civilian practice.

Discovery of Pyrogens. This landmark in parenteral therapy has never received just recognition of its importance. In 1923 Florence Seibert[16] announced the discovery of heat-stable products from bacteria growing in distilled water. By taking certain precautions against the contamination of fluids with pyrogens, injections of solutions or blood can be given to human beings with a minimal incidence of chills and fever. This finally allayed the doubts as to the innocuousness of sodium citrate, so that the delayed method of transfusion became more widely employed. Another result was the commercial production of solutions for parenteral therapy and preservative mixtures for blood, which has contributed materially to the increased use of blood transfusion in the United States.

Preservation of Blood. Within two years after the introduction of sodium citrate as an anticoagulant, Rous and Turner of the Rockefeller Institute published the pioneer studies[17] on the preservation of blood for transfusion. Almost immediately the results were applied by Robertson who conceived and operated the first blood bank. He collected stores of blood in casualty clearing stations of the British Army and transfused wounded soldiers. His report was published in 1918.[18]

The principle of the blood bank was temporarily forgotten after World War I, to receive a revival in Russia by Yudin who used cadaver blood in 1933.[19] The first blood bank in the United States was organized in 1936 at Cook County Hospital in Chicago;[20] since then, the idea has spread with great rapidity in this country and elsewhere. In almost every institution in which a bank has been established the number of blood transfusions has been greatly increased so that this development has resulted in more nearly supplying the needs of patients for transfusion than ever before in the history of medicine.

In World War II the use of preserved blood was greatly accelerated. In Great Britain blood banks were organized for the treatment of civilians injured in air raids. The British Army Blood Transfusion Service furnished preserved blood for the troops in combat in Europe and Africa. Later, the Army of the United States operated transfusion services in the European Theater of Operations and the Mediterranean Theater. Approximately a third of a million transfusions of preserved whole blood were given

time. The operation was crude and difficult. Transfusion was performed either by artery-to-vein anastomosis or by some modification of the syringe method. There was no effective way of preventing coagulation of the blood. Probably about 40 per cent of the transfusions resulted in intravascular hemolysis, with a high mortality.

THE LAST 50 YEARS

The Period of Application. *The Discovery of the Four Blood Groups.* In 1900 Landsteiner[7] announced his discovery of various patterns of agglutinogens and agglutinins in human individuals and reported three blood groups, some of which were mutually incompatible in blood transfusion. Decastello and Sturli[8] described the fourth blood group two years later. Ottenberg[9] and his associates were the first to apply this knowledge to human transfusion. In 1907 Janský[10] reported his numerical classification of the four groups in a Bohemian journal which attracted little notice at the time. In the United States, Moss[11] announced a somewhat similar nomenclature independently in 1910.

During the first decade of the twentieth century interest was again greatly stimulated in blood transfusion because the major cause of fatalities had been discovered and the methods of prevention had been demonstrated. Many attempts were made to improve the mechanics of the procedure. These resulted in the techniques of Carrel, of Crile, and of Bernheim in artery-to-vein anastomosis, and the paraffin tube method of Kimpton and Brown. But again, these advances did not affect the average patient who needed a transfusion. The techniques were still so difficult as to require the services of highly skilled persons in an operating room. The vessels of the donor and recipient were usually ligated. It is safe to state that only a few transfusions were performed in comparison with the need.

Introduction of Sodium Citrate. Perhaps next in importance to the discovery of the four blood groups was the introduction of sodium citrate as an anticoagulant in 1914 by Hustin[12] in Belgium, Agote[13] in Argentina, and Lewisohn[14] and Weil[15] in the United States. This advance made possible the addition of a simple, inexpensive, relatively nontoxic chemical substance to the blood which permitted a much-needed simplication of the procedure of transfusion. The collection of blood could be separated from its injection by minutes or hours. The presence of the donor was not required in the same room with the recipient. This permitted, for the first time, what we have termed "delayed transfusion" (p. 516). The technique of blood transfusion was immediately simplified so that

transfusion. The knowledge of isosensitization has led to new techniques for testing blood compatibility before transfusion and in the diagnosis and treatment of hemolytic disease of the newborn. Although the overall safety of blood transfusion has been increased but little by these discoveries, a new vista of research in immunology and genetics has been opened which may have widespread effects on the understanding of some disease processes.

Summary. The clinical experience of over 300 years has led to the conclusion that the great majority of patients who need blood require the erythrocytes particularly. The simplest method for supplying these is by whole blood transfusion. The procedures are now known and practical to supply most patients in the country with transfusions of safe blood.

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in the ETOUSA alone. About half of this amount was flown from the continental United States. The United States Navy operated a transfusion service which transported blood by airplane from the United States to the Pacific Ocean Areas.

The experience in wartime had a profound effect on blood transfusion in civilian practice. Many physicians who served in the armed services acquired knowledge and skill in the practice which they carried back to civilian life. In Great Britain a national transfusion service was organized to serve civilian needs. At the present writing, the American National Red Cross is initiating a program to supply preserved blood to communities where it is needed.

Blood Plasma. Blood serum and plasma had been used therapeutically in the United States for many years, probably as an outgrowth of the "serum center" in which convalescent serum was processed. As early as 1936 John Elliott[21] reported the use of blood plasma, and Strumia[22] stated that his experience with it began in 1927. When blood banks developed with the consequent supply of plasma as a byproduct from outdated blood, interest in the clinical use of plasma increased. Most of the pioneer work was done in 1939. Preliminary experiments and observations seemed to indicate that hemorrhagic shock could be treated successfully with transfusions of plasma. A short-lived wave of enthusiasm for plasma therapy ensued in the United States which was dampened about 1943–1944 by the extensive experience of the armed forces in the treatment of wounded men. Gradually the clinical and experimental evidence became overwhelming to indicate that plasma transfusions were principally of value as a first-aid measure in the treatment of hemorrhagic shock, and that plasma would not supplant transfusions of whole blood. The treatment of chronic hypoproteinemia with plasma has also proved disappointing.

Plasma Fractions. An outgrowth of the processing of plasma on a huge scale for the armed forces was the physicochemical fractionation of human plasma in large quantities for therapeutic use This was done primarily by Cohn and his coworkers[23] under the auspices of the National Research Council and the Office of Scientific Research and Development. The clinical evaluation of the plasma fractions is still under way as a project of the American National Red Cross.

The Rh Factor. The discovery of the Rh antigens by Landsteiner and Wiener[24] in 1940 and the demonstration of their relation to the cause of erythroblastosis fetalis by Levine and his colleagues[25] have opened a new field of immunology closely related to blood

transfusion. The knowledge of isosensitization has led to new techniques for testing blood compatibility before transfusion and in the diagnosis and treatment of hemolytic disease of the newborn. Although the overall safety of blood transfusion has been increased but little by these discoveries, a new vista of research in immunology and genetics has been opened which may have widespread effects on the understanding of some disease processes.

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CHAPTER 2

Therapeutic Choice of Blood, Blood Derivatives and Plasma Substitutes

By John B. Alsever

In the tables and text of this chapter are presented the indications for and the relative therapeutic merits of blood, blood derivatives and certain macromolecular substances, administered by various routes. A detailed discussion of their clinical use in various conditions, their preparation, and administration will be found in subsequent chapters.

PRODUCTS FOR USE INTRAVENOUSLY

Whole Blood

Whole blood, fresh, stored, and preserved, is more useful generally than any of the blood fractions or plasma substitutes. In any general hospital service whole blood is indicated in from 60 to 90 per cent of the transfusions. Unfortunately, transfusions of whole blood (and red cell suspensions) require grouping and cross-matching and are, therefore, the most difficult to administer safely, since error may result in serious or fatal reactions. The use of the usual anticoagulant, sodium citrate, does not alter the blood in any way which is of clinical importance. Neither the loss of free calcium nor the presence of the citrate ion decreases the clinical usefulness of whole blood. The danger of producing tetany from the administration of the citrate which is contained in blood or plasma is neither great nor serious (p. 296).

Fresh Blood. The blood must be administered to the patient within a few hours after collection from the donor. It should be

placed in the refrigerator at once and must be kept cold (5° to 10° C.) during the interval, if it is to be considered as fresh blood. Under these conditions the blood, regardless of the anticoagulant solution used, will be as effective as in an immediate transfusion. Fresh whole blood contains the maximum of all components of the donor's blood. If blood cannot be adequately refrigerated as set forth above, it should be administered at once. The use of unrefrigerated blood, except for immediate transfusion, is not recommended.

Preserved Blood. When blood, in a solution containing an anticoagulant and dextrose, has been kept at 5° to 10° C. for longer than a few hours, progressive changes have occurred and it should be considered as *preserved blood.* These changes take place at different rates, however, so that preserved blood may be as effective clinically as fresh blood for most purposes for as long as four weeks. The leukocytes and platelets disintegrate fairly rapidly and the majority have disappeared by the fifth day of storage. Complement and prothrombin decrease rather slowly and show a significant change after the tenth to fourteenth day. The range of usefulness for red cells varies from fourteen to thirty days, depending on the composition and volume of the anticoagulant solution. Several satisfactory preservative solutions for whole blood, together with their uses, advantages, and limitations, are described in detail in Chapter 13. The transmission of certain diseases by blood transfusion is much less likely if the blood has been stored for a period of time (Chap. 12).

Stored Blood. When blood has been collected in sodium citrate solution only and has been held at 5° to 10° C. for more than a few hours, progressive changes have occurred, and it should be considered as *stored blood.* Unlike preserved blood, degenerative changes develop rapidly in the erythrocytes of stored blood. Because of the rapid deterioration of red cells, stored blood should not be used for whole blood transfusion after the fifth day. Stored blood may be considered equivalent to preserved blood for the first three days of storage (Chap. 13).

Citrated Normal Blood Plasma or Serum

Plasma may be prepared and stored in any one of several ways as described in Chapters 16, 17, and 18. It contains most of the sodium citrate which was added to the whole blood. The range of usefulness of plasma depends on the method of its preparation and storage. For example, plasma, fresh or stabilized by freezing or drying from the fresh state, is required in certain conditions where its unstable components are essential.

Liquid Plasma. Liquid plasma containing 5 per cent dextrose, stored at room temperature, may be used to treat shock or hypoproteinemia for at least three years. Under these conditions complement and prothrombin content deteriorate appreciably seventy-two hours after the collection of the blood (ten days, if kept at 5° to 10° C.) and the antibody content ordinarily has decreased significantly after six months of storage. The presence of 5 per cent dextrose in liquid plasma prevents, to a great extent, the precipitation of fibrin during storage at room temperature. Fibrin will form if plasma is stored at 5° to 10°C., regardless of whether or not dextrose has been added (Chap. 16).

Frozen Plasma. If blood plasma has been separated from the cells and brought to the frozen state within seventy-two hours after collection of the blood, it may be stored almost indefinitely and will not show deterioration of complement, prothrombin, or antibodies. The temperature of storage should be below −20° C. and the plasma should be used promptly after thawing. It should be thawed at 37° C. in a water bath. Reliquified frozen plasma, if sterile, has the same storage characteristics as liquid plasma with one exception. Frozen plasma may or may not contain 5 per cent dextrose which has been found necessary to stabilize the fibrinogen during storage at room temperature (Chap. 17).

Dried Plasma. Plasma, which is dried after shell freezing within seventy-two hours after collection, is stable for at least five years if the container maintains an airtight seal. A full content of the labile constituents will be present if the plasma is used promptly after restoration to the liquid state with 0.1 per cent solution of citric acid. Dried plasma must be administered within one hour after being put into solution (Chap. 18).

Therapeutic Categories of Plasma. There are three therapeutic categories of plasma:

Fresh Plasma. This contains maximum amounts of all components originally present in the donor's plasma. It is either liquid plasma less than seventy-two hours old, or frozen or dried plasma which is brought to the frozen state within seventy-two hours after bleeding and used promptly after reliquification.

Plasma for Immune Therapy. This may be fresh plasma or liquid plasma stored for short periods, but less than six months, at room temperature (Chap. 19).

Plasma for the Treatment of Shock or Hypoproteinemia. For this purpose plasma may have been stored as long as three years at room temperature in the liquid state, or may be fresh liquid, frozen or dried.

RESUSPENDED RED CELLS

In the preparation of plasma, the red cells are by-products which can be used readily for transfusions. When obtained from blood collected in citrate solution only, the erythrocytes can be used up to five days after collection. They may or may not be diluted with varying amounts of isotonic saline, depending on the need to limit the volume of fluids given intravenously. However, preservative solutions also have been developed for resuspending red cell residues so that they may be used up to twenty-one days of storage. The blood group of the erythrocytes must be known and crossmatching is essential, unless proven Group O cells are used (Chap. 21).

SERUM ALBUMIN

Serum albumin is a fraction of normal human plasma. It is unusually stable in the liquid form and may be prepared as a 5 per cent (isotonic) solution or a 25 per cent (five times concentrated) solution. The latter has the same viscosity as whole blood and 100 ml. is the osmotic equivalent of 500 ml. of plasma (Chap. 22).

ANTIHEMOPHILIC GLOBULIN

This is also a plasma fraction and, at the present time, is usually prepared in combination with the fibrinogen fraction, although it can be isolated by a special technique. The intravenous injection of the fibrinogen fraction reduces the bleeding time of hemophiliacs for several hours. Thereafter the bleeding time slowly returns to its former value. The globulin is apparently quite unstable; it requires very careful processing of the plasma during fractionation to avoid denaturation, and it must be stored in the dried state (Chap. 22).

GELATIN SOLUTIONS

Ossein Gelatin. Solutions of animal gelatin, like other macromolecular plasma substitutes, must be prepared especially for intravenous use. Gelatin is of limited value in the treatment of shock and is not recommended for the treatment of hypoproteinemia. It is, however, the best of the plasma substitutes. It is appreciably superior to solutions of crystalloids but is distinctly inferior to blood and its derivatives. There are clinically important disadvantages which make gelatin solutions less desirable to use. namely, its high viscosity at room temperature, its rapid urinary excretion, and its interference with subsequent blood grouping

and crossmatching by causing excessive rouleau formation unless special techniques are employed (Chap. 23).

Fish Gelatin (Isinglass). Solutions of isinglass have been employed by Canadian workers with results which are approximately equivalent to those obtained with animal gelatin (Chap. 23).

Other Plasma Substitutes

Other substances which have been used in solution as plasma substitutes are either experimental or not so satisfactory as gelatin solutions. Among these are:

Globin. This is prepared from human hemoglobin. Globin solutions have been used experimentally with success in the treatment of shock (Chap. 21).

Acacia. In addition to the disadvantages of gelatin, acacia is deposited in the tissues of the body and at least is potentially harmful (Chap. 23).

Pectin. This plasma substitute is also deposited in tissues, but to a lesser extent than acacia (Chap. 23).

Methylcellulose. This agent has been used experimentally in animals as a plasma substitute but produces kidney damage (Chap. 23).

Hemoglobin. Solutions of human hemoglobin have been used experimentally in the treatment of shock. However, the use of this agent is not justified in view of the known facts regarding its toxicity (Chap. 21).

RELATIVE MERITS OF PRODUCTS FOR USE INTRAVENOUSLY

Shock

The fundamental physiologic disturbance in shock is the loss of circulating blood volume, and the chief therapeutic measure is the physiologic replacement of this loss to restore and maintain adequate oxygenation of the body tissues (Chap. 3).

Hemorrhagic (Traumatic) Shock. This type of shock is due to the loss of blood externally or into the injured tissues or the body cavities. The presence of trauma implies hemorrhage.

First Choice—Whole Blood, Fresh or Preserved. Physiologically, whole blood replaces that which has been lost, both red cells and plasma, and the blood volume and oxygen-carrying capacity of the blood can be restored and maintained. An approximately normal volume of erythrocytes also assists in holding the plasma proteins in the circulation.

Second Choice—Plasma, Liquid, Frozen, or Dried. Plasma replaces the lost blood volume but the effect is not so well sustained as

Resuspended Red Cells

In the preparation of plasma, the red cells are by-products which can be used readily for transfusions. When obtained from blood collected in citrate solution only, the erythrocytes can be used up to five days after collection. They may or may not be diluted with varying amounts of isotonic saline, depending on the need to limit the volume of fluids given intravenously. However, preservative solutions also have been developed for resuspending red cell residues so that they may be used up to twenty-one days of storage. The blood group of the erythrocytes must be known and crossmatching is essential, unless proven Group O cells are used (Chap. 21).

Serum Albumin

Serum albumin is a fraction of normal human plasma. It is unusually stable in the liquid form and may be prepared as a 5 per cent (isotonic) solution or a 25 per cent (five times concentrated) solution. The latter has the same viscosity as whole blood and 100 ml. is the osmotic equivalent of 500 ml. of plasma (Chap. 22).

Antihemophilic Globulin

This is also a plasma fraction and, at the present time, is usually prepared in combination with the fibrinogen fraction, although it can be isolated by a special technique. The intravenous injection of the fibrinogen fraction reduces the bleeding time of hemophiliacs for several hours. Thereafter the bleeding time slowly returns to its former value. The globulin is apparently quite unstable; it requires very careful processing of the plasma during fractionation to avoid denaturation, and it must be stored in the dried state (Chap. 22).

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Second Choice—Plasma, Liquid, Frozen, or Dried. Plasma replaces the lost blood volume but the effect is not so well sustained as

THERAPEUTIC CHOICE FOR USE INTRAVENOUSLY

A = First Choice, B = Second Choice, C = Third Choice, D = Fourth Choice, E = Fifth Choice

	Whole Blood		Plasma			Resuspended Red Cells	Human Serum Albumin	Antihemophilic Globulin	Gelatin
	Fresh	Preserved	Liquid	Frozen	Dried				
Shock, hemorrhagic	A	A	B	B	B	D	C		E
[illegible]	B	B	A	A	A		C		D
[illegible]	B	B	C	C	A[4]		A[4]		D
Shock, medical[3]	B	B	A	A	A		C		D
Anemia, acute	A	A				B			
Anemia, chronic	B	C				A			
Leukopenia[2] or thrombocytopenia[2]									
Hypoproteinemia, acute[1]	D	D	C	C	B[4]		A[4]		
Hypoproteinemia, chronic[1]									
[illegible]	B	B				A			
[illegible] deficiency	B	A[5]	A[6]	A[7]	A[7]				
Hemophilia	B	C	B[6]	B[7]	B[7]			A	
Immunotherapy	B	B	A[8]	A	A				

[1] Initial treatment
[2] No treatment with blood recommended
[3] Several causes (cf. text)
[4] Concentrated
[5] Stored less than 10 days
[6] Fresh liquid plasma
[7] Plasma quickly stabilized after collection
[8] Plasma stored less than 3 months

when whole blood is given. However, in *massive hemorrhage* red cells or whole blood are essential within a few hours if life is to be sustained.

Third Choice—Serum Albumin. The physiologic effect of serum albumin closely approaches that of plasma. However, one should always realize that *only* albumin is replaced and that if the loss of plasma has been severe, a deficiency of other blood proteins may exist, especially if blood or plasma loss continues over a period of time. Concentrated albumin should be used with caution in the presence of dehydration (pp. 42 and 451).

Fourth Choice—Resuspended Red Cells. Physiologically resuspended red cells will restore the oxygen-carrying capacity of the blood, tend to hold the plasma protein in the circulation, and the added volume of the diluting or preserving solution will assist temporarily in restoring the blood volume. Plasma always should be given to bring the protein level to normal.

Fifth Choice—Gelatin Solution. Although gelatin solution will restore blood volume, the effect is temporary (forty-eight to seventy-two hours) and the gelatin content of the plasma requires special methods for subsequent compatibility tests for transfusions of blood (p. 457).

Sixth Choice—Other Plasma Substitutes. These other plasma substitutes are either experimental or inferior to gelatin (see discussion of products). However, preliminary studies with human globin (p. 442) indicate that it may come to occupy a place with albumin in the treatment of shock.

Burn Shock, Crush Syndrome. Burn shock is due to the initial loss of plasma externally and into the injured tissues, with resulting hemoconcentration. In crush syndrome there is an initial loss of whole blood but the loss of plasma soon predominates. Replacement of lost blood volume should be accomplished with:

First Choice—Plasma, Liquid, Frozen, or Dried. Plasma physiologically replaces that which has been lost, and has the advantage of being retained. Large quantities usually are needed during the first forty-eight hours (Chap. 3), after which whole blood is required to correct the anemia.

Second Choice—Whole Blood, Fresh or Preserved. Whole blood most nearly approaches plasma in physiologic replacement and, in view of the eventual anemia, may be preferable, unless hemoconcentration is marked (Chap. 3).

Red cell suspensions are not recommended for the treatment of the anemia which develops in burned patients because a lack of plasma proteins usually coexists.

Third Choice—Serum Albumin. Serum albumin will largely re-

THERAPEUTIC CHOICE FOR USE INTRAVENOUSLY

A = First Choice , B = Second Choice , C = Third Choice , D = Fourth Choice , E = Fifth Choice

SHOCK, HEMORRHAGIC
SHOCK, BURN[1] OR CRUSH SYDROM[4]
SHOCK WITH CEREBRAL HEMORRHA
SHOCK, MEDICAL[3]
ANEMIA, ACUTE
ANEMIA, CHRONIC
LEUKOPENIA[2] OR THROMBOCYTOPENIA[2]
HYPOPROTEINEMIA, ACUTE
HYPOPROTEINEMIA, CHRONI-
CARBON MONOXIDE POISONING OR METHEMOGLOBINEMIA
COMPLEMENT OR PROTHROMBIN DEFICIENCY
HEMOPHILIA
IMMUNOTHERAPY

[1] Initial treatment
[2] No treatment with blood recommended
[3] Several causes (cf. text)
[4] Concentrated
[5] Stored less than 10 days
[6] Fresh liquid plasma
[7] Plasma quickly stabilized after collection
[8] Plasma stored less than 3 months

disease, diabetic coma following dehydration, cholera, and infant diarrhea. One must be certain, however, that the condition is not merely one of primary shock in which replacement by crystalloid solutions is sufficient and in which the use of blood or blood derivatives is contraindicated (Chap. 3). Replacement of the lost blood volume should be accomplished with:

First Choice—Plasma, Liquid, Frozen, or Dried. Physiologically, plasma replaces that which has been lost. Considerably more care is necessary in the treatment of "medical" shock than in that following trauma if circulatory overload is to be avoided, especially when impaired cardiac function or pulmonary disease coexist (pp. 270 and 435).

Second Choice—Whole Blood, Fresh or Preserved. Whole blood most nearly approaches plasma as a physiological replacement. In some cases the added volume of certain types of preserved blood may not be desirable for the reason given before.

Third Choice—Serum Albumin. Somewhat arbitrarily serum albumin is rated as third choice because, in the concentrated form, it may be contraindicated on the basis of dehydration or the danger of too rapidly increasing the blood volume (pp. 451 and 270).

Fourth and Fifth Choice—Gelatin and Other Plasma Substitutes. These plasma substitutes are so rated for the same reasons as given for hemorrhagic shock.

ANEMIA

Acute Anemia. This is always due to the rapid loss of whole blood, except in those relatively uncommon conditions in which it results from the rapid intravascular destruction of red cells (hemolytic anemia) due to disease or to the effect of a toxic substance. Replacement should be accomplished by:

First Choice—Whole Blood, Fresh or Preserved. Whole blood physiologically replaces both the red cells and plasma which have been lost, except in the case of the hemolytic anemias (see Second Choice).

Second Choice—Resuspended Red Cells. Physiologically, resuspended red cells replace the oxygen-carrying capacity of the blood, and the added volume of the diluting solution temporarily aids in restoring the blood volume. Plasma should always be given to bring the protein level to normal.

In hemolytic anemias, resuspended red cells rate as first choice because there has been no loss of plasma.

Chronic Anemia. The chronic type of anemia may be due to any of several causes such as repeated small hemorrhages, iron deficiency, blood dyscrasias, chronic infections, or malignancy. Replacement should be accomplished by:

place the lost protein. However, because of the marked loss of plasma, the disadvantages of albumin are especially pertinent and concentrated material is contraindicated (see *Hemorrhagic Shock*, p. 13).

Fourth and Fifth Choices—Gelatin and Other Plasma Substitutes. These plasma substitutes are so rated for the same reasons as given for hemorrhagic shock (p. 13).

Shock With Cerebral Damage. Although this is traumatic shock, it differs in that the loss of blood and plasma into the injured brain tissues may cause edema which can prove fatal. Replacement of lost blood volume should be accomplished with:

First Choice—Concentrated Blood Plasma or Serum Albumin. This blood plasma or serum albumin physiologically replaces the blood volume, and the hypertonicity may draw fluid from the tissues into the circulating blood, thereby tending to minimize or prevent the development of edema in the injured brain tissue. Dried plasma can be reconstituted to from one-fourth to one-half its original volume, or liquid plasma can be similarly concentrated by repeated freezing, thereby providing a two to four times concentrated solution of plasma proteins (p. 398). Concentrated serum albumin (25 per cent solution) may also be employed. The use of such concentrated protein solutions should be based solely on the need to replace lost blood volume. Other agents or methods should be employed when the primary purpose of therapy is to counteract cerebral edema.

Second Choice—Whole Blood, Fresh or Preserved. Since the condition is a type of hemorrhagic (traumatic) shock, the lost whole blood should be replaced. Whole blood would, therefore, be first choice, except for the desire to also minimize or prevent the edema of injured brain tissue. Preserved blood may be less desirable than fresh blood because of its increased volume. This, however, should not be of great importance if a low volume preservative solution, such as ACD (acid-citrate-dextrose solution), is employed. If anemia is found to exist after treatment with concentrated protein solution and minimal dilution is desired, a concentrated suspension of red cells may be employed to advantage.

Third Choice—Isotonic Plasma. This may be undiluted liquid, frozen, or dried plasma reconstituted to its original volume. It most nearly approaches whole blood in physiologic replacement.

Fourth and Fifth Choice—Gelatin and Other Plasma Substitutes. These plasma substitutes are so rated for the same reasons as given for hemorrhagic shock.

"Medical" Shock. This is due to a loss of circulating blood volume (protein loss) which occurs in such conditions as Addison's

Second Choice—Whole Blood, Fresh or Preserved. Although the antibody content of whole blood is the same as that of the plasma, the whole blood must be obtained from a donor whose blood is compatible with that of the recipient and the risk of reaction is greater. Further, the administration of the red cells is ordinarily unnecessary.

Acute and Chronic Hypoproteinemia

A low concentration of plasma protein may be encountered during convalescence from burn shock and occasionally from hemorrhagic shock, or as a manifestation of such chronic conditions as malnutrition, hepatic cirrhosis, renal disorders, and gastrointestinal diseases.

Acute Hypoproteinemia. Correction can be accomplished by:

First Choice—Concentrated Blood Plasma or Serum Albumin. With concentrated solutions the required protein can be given in a smaller fluid volume and the administration of larger amounts is facilitated. In the use of albumin, although it is quite satisfactory to reestablish normal intravascular osmotic pressure, care should be observed that replacement of other plasma proteins is not also indicated. The use of concentrated solutions of protein causes a rapid increase in blood volume and therefore they must be employed with caution where this may cause circulatory overload (p. 270).

Second Choice—Plasma, Liquid, Frozen, or Dried. Plasma furnishes replacement of all blood proteins. However, adequate replacement with isotonic plasma may require the daily use of volumes too large to be practicable.

Third Choice—Whole Blood, Fresh or Preserved. Whole blood is third choice because the red cells often are not needed, the blood must be compatible, and, as with isotonic plasma, very large amounts may be needed. If, however, an anemia coexists, whole blood becomes *first choice* until this has been corrected. Here again, fresh blood, or blood preserved in a low volume solution, usually is desirable to minimize the total volume of fluid administered intravenously.

Chronic Hypoproteinemia. The use of transfusions of whole blood, blood plasma, or serum albumin are not recommended. The amounts required are so large as to be ordinarily impractical. Correction can be accomplished by:

First Choice—Protein Feeding. This method is much the most efficacious if the patient can eat.

Second Choice—Amino Acid Solutions Intravenously. Several preparations of amino acid solutions are available which may be employed satisfactorily intravenously.

First Choice—Resuspended Red Cells. Red cells most nearly provide physiologic replacement of that which has been lost. This, of course, is predicated on the fact that, in the majority of such cases, there is no concomitant decrease in the plasma protein level. If, however, hypoproteinemia occurs, whole blood, fresh or preserved, becomes *first choice.*

Second Choice—Whole Blood, Fresh or Preserved. The red cells must be replaced even though plasma is also given without any specific indication. Whole blood becomes the logical first choice if the plasma proteins are diminished. In any event many chronically ill persons can well afford this intravenous supplement if not in actual need of protein replacement.

LEUKOPENIA AND THROMBOCYTOPENIA

These conditions may be due to a variety of causes, both known and unknown. Transfusion replacement therapy is, at best, quite unsatisfactory because of the large fluid volume needed to provide even a relatively small number of platelets or leukocytes. For example, to raise the leukocyte count from 2000 per cu. mm. to 5000, one would have to add 3000 white cells per cu. mm. Since at least 500 to 1000 red cells are given for each white cell, the erythrocyte count would be increased by 3,000,000 per cu. mm. at the same time, a transfusion of three-fifths of the normal blood volume. In a 70-kilogram patient, this would be on the order of a 3500-ml. blood transfusion. Replacement may be attempted by:

First Choice—Fresh Whole Blood. Fresh whole blood contains the maximum number of leukocytes and thrombocytes. The use of the so-called "leukocytic cream" is not a practicable procedure and is of doubtful added therapeutic value.

Second Choice—Preserved Blood. Both leukocytes and thrombocytes disintegrate rapidly as the blood ages (p. 308) so that blood more than two or three days old would be without any possible value.

IMMUNOTHERAPY

The use of normal human blood, serum, or plasma, as well as that collected from persons convalescing from a specific disease, has been rather widely employed intravenously, with varying success in the treatment of a number of infectious diseases. More recently, hyperimmune human serum has been prepared and employed with considerable success in pertussis (Chap. 19; see also *Immune Therapy*, p. 20).

First Choice—Plasma, Frozen or Dried. Rapidly stabilized frozen or dried plasma is the product of choice. Plasma stored in the liquid state also may be used if it is less than three months old.

Second Choice—Plasma, Fresh Liquid, Frozen, or Dried. Plasma contains the required globulin and is easier to administer than a blood transfusion if it is available. Stored liquid plasma may also be employed if less than six months old (p. 350).

Third Choice—Whole Blood, Fresh or Preserved. Whole blood also corrects the deficiency but must be compatible. Whole blood becomes *first choice* if an associated anemia is present.

PRODUCTS FOR USE INTRAMUSCULARLY

WHOLE BLOOD

Fresh, stored, or preserved whole blood (p. 7) may be employed (see discussion of indications) and need not be of the same blood group as the recipient.

THERAPEUTIC CHOICE FOR USE INTRAMUSCULARLY

A = First Choice *B = Second Choice* *C = Third Choice*

	WHOLE BLOOD		PLASMA			IMMUNE SERUM GLOBULIN
	fresh	preserved	liquid	frozen	dried	
IMMUNE THERAPY	C	C	B[1]	B	A[3]	A
HEMOPHILIA	C	C	B[2]	B	A	

[1] Plasma stored less than 3 months
[2] Fresh
[3] Concentrated

CITRATED BLOOD PLASMA OR SERUM

Either liquid plasma, frozen plasma, or the dried form (p. 420) may be used (see discussion of indications).

IMMUNE SERUM GLOBULIN

Immune human serum globulin is a fraction of plasma and contains nearly all the antibodies present in the original blood. It is used intramuscularly and almost never produces an appreciable reaction. Although it would be highly desirable clinically, it has not been possible as yet to prepare this material in a form which can be employed intravenously without rather severe reactions. The immune substances are concentrated approximately twenty-five times in the standard preparation. It can be prepared from pooled *normal* adult plasma, convalescent plasma or from hyperimmune plasma (p. 449).

Carbon Monoxide Poisoning and Methemoglobinemia

Certain types of poisons unite with the hemoglobin in the red cells producing compounds in which the oxygen is so firmly bound that the normal exchange of oxygen and carbon dioxide in the tissues does not take place. Death may result from an inadequate supply of oxygen unless normal red cells can be quickly transfused. Therapy should be accomplished by:

First Choice—Resuspended Red Cells. These resuspended red cells permit the most rapid possible replacement and plasma protein is not needed.

Second Choice—Whole Blood, Fresh or Preserved. Again the use of a preservative solution of small volume may be preferable because the number of erythrocytes per unit of injected solution is higher.

Deficiency of Complement or Prothrombin

Deficiency of complement is sometimes seen in severe acute infectious diseases and in the development of allergic phenomena such as serum sickness and nephritis after an acute streptococcal infection. The correction of this deficiency may produce a striking improvement in the clinical condition of the patient (p. 426).

Deficiency of prothrombin sometimes occurs in the hemorrhagic syndrome observed in the newborn and in cases with jaundice due to impaired liver function. When a deficiency exists, the replacement of prothrombin is characteristically followed by cessation of abnormal bleeding.

Therapy in these two deficiencies should be accomplished by:

First Choice—Plasma, Fresh Liquid, Frozen, or Dried. The use of plasma enables the deficiency to be corrected easily without the necessity of finding compatible blood. The red cells often are not needed.

Second Choice—Whole Blood, Fresh or Preserved. The latter should be employed only within seven to ten days after collection (p. 309). Obviously, if an anemia coexists, whole blood becomes *first choice.*

Hemophilia

Hemophilia, at least in the majority of cases, apparently is due to a deficiency of a specific globulin which recently has been found to be associated with the fibrinogen fraction of plasma (p. 448). It has long been known that the administration of blood or plasma would arrest the abnormal bleeding in most cases.

Therapy should be accomplished by:

First Choice—Antihemophilic Globulin. This product corrects the deficiency and is easier to administer than a transfusion if it is available.

Third Choice—Whole Blood, Fresh or Preserved. The blood should be less than seven to ten days old when prothrombin is required. The local reaction is greatest when whole blood is used.

PRODUCTS FOR TOPICAL USE

Citrated Blood Plasma

Either fresh liquid plasma, frozen or dried plasma (p. 9) may be employed.

Fibrinogen and Thrombin

These are both obtained from the fractionation of human plasma. They are prepared in powdered form and each is put into solution separately just prior to use. Although it is believed by many that human fibrinogen and thrombin are preferable for use because they are species-specific proteins, it should be pointed out that quite satisfactory thrombin appears to have been derived from animal plasma (p. 458).

THERAPEUTIC CHOICE FOR USE TOPICALLY

A = First Choice B = Second Choice

	PLASMA			FIBRINOGEN AND THROMBIN	FIBRIN		RED CELL PASTE OR POWDER	GELATIN FOAM
	liquid	frozen	dried		films	foams		
REPAIR OF TISSUES	B	B	B	A	A			
HEMOSTASIS				B		A		A
WOUND DRESSINGS	B	B	B				A	

Fibrin Products

Human fibrin, formed by combining fibrinogen and thrombin, can be prepared for use in several ways (p. 448).

Foams. Fibrin foams are light sponge-like preparations. For use, a piece of foam is saturated in a solution of thrombin, after which it can readily be cut and molded into any desired size and shape. It should be noted that soluble cotton or gelatin foam and animal thrombin appear to be equally as satisfactory (p. 458).

Films. The thickness, pliability, and other characteristics of fibrin films can be controlled within satisfactory limits, depending on the use to which the film is to be put. Like foams, the films are stored in the dry state. To restore pliability at the time of use they are soaked in saline solution. Polyethylene films also show some promise (p. 458).

RELATIVE MERITS OF PRODUCTS FOR USE INTRAMUSCULARLY

IMMUNE THERAPY

In the treatment of an acute infection, it is far more desirable to administer blood and plasma or serum intravenously because of the promptness of the therapeutic effect and the ease of employing the large amounts sometimes required. If reactions occur, they are usually mild. In the prophylaxis of exposed individuals, however, intramuscular administration is preferable because it is simpler and rapid availability of antibodies is not required.

First Choice—Concentrated Dried or Frozen Plasma, or Immune Serum Globulin. Concentrated plasma should be used in diseases in which immuno-therapy is effective. The dried form can be given in three or four times the original concentration and liquid plasma can be concentrated by repeated freezing (p. 398). The resultant reduction in the volume of plasma required for injection is advantageous. However, the use of plasma customarily produces some local reaction. If available, immune serum globulin is the first choice for diseases in which it is effective because it provides twenty-five times the concentration of antibodies in the original blood. Local reactions are nearly nonexistent (p. 449).

Second Choice—Isotonic Plasma, Liquid Less Than Three Months Old, or Frozen. Although full antibody content is present, these products are not concentrated and the amount required is much larger. A local reaction at the site of injection also is customarily present.

Third Choice—Whole Blood, Fresh or Preserved. While the therapy is just as effective, the local reaction is much more pronounced than with plasma.

BLOOD DYSCRASIAS

The use of intramuscular therapy is limited to those diseases in which a hemorrhagic tendency is due to a deficiency of some element normally present in blood. Factors which can be supplied include prothrombin and antihemophilic globulin (pp. 309 and 350). Therapy should be accomplished by:

First Choice—Concentrated Dried or Frozen Plasma. This provides the required dose in one-third to one-fourth the volume required when isotonic plasma is used. A local reaction usually follows administration.

Second Choice—Isotonic Plasma, Liquid or Frozen. Although as effective as concentrated plasma, the use of isotonic material requires the administration of a much larger amount. Local reaction is customarily present. Liquid plasma must be fresh when prothrombin is required. Liquid plasma up to six months old is reported to be effective in hemophilia (pp. 19 and 350).

foam or cotton with thrombin, although not essential, enhances the results obtained. These products are absorbed completely without local reaction and have been found especially uselul in neurosurgery (pp. 448 and 458).

Second Choice—Fibrinogen and Thrombin Solutions or Powder. These substances also produce rapid hemostasis at small bleeding points. Their use is less satisfactory, however, because there is no preformed matrix to aid in clot formation.

Wound Dressings. The topical application of a protein-rich substance materially enhances wound healing and is of particular value in chronic lesions, such as indolent ulcers, or severe burns (p. 440).

First Choice—Red Cell Paste, Powder, or Ointment. All three ol these products give satisfactory results; the cells are usually available as a waste product of a blood bank, and the paste, powder or ointment lends itself readily to topical use.

Second Choice—Plasma, Liquid, Frozen or Dried. Although the results are comparable to those with red cells, plasma is less economical and less satisfactory to use.

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Experimental Products. Fibrin plastics have been produced experimentally in the form of tubes which can be used in surgery for drainage or repair of tubular structures. The characteristics of the fibrin can be varied by changes in the plasticizer used, and it appears possible to produce a great variety of fibrin plastics. Again, polyethylene products may be equally useful (p. 458).

RED CELL PASTE, POWDER, OR OINTMENT

Red cell paste is prepared by allowing the sterile red blood cell mass remaining to stand in the refrigerator for two weeks or longer after the aspiration of plasma. Such material has the characteristics of a thick gelatinous paste which lends itself to topical application. The red cell residue can also be dried and used as a powder or incorporated in an ointment base for topical application (p. 440).

RELATIVE MERITS OF PRODUCTS FOR TOPICAL USE

The topical use of blood derivatives (or suitable substitutes) in the repair of tissues in surgery, including hemostasis, are of demonstrated value, particularly in neurosurgical procedures and in affixing skin grafts. Potentially there are several other opportunities for the use of these products (Chaps. 22 and 23).

Repair of Dura Mater. *First Choice—Fibrin Film, Polyethylene Film.* Fibrin gives adequate closure, produces little local reaction, and disappears as it is replaced by new tissue without the formation of adhesions. The use of polyethylene film may result in adhesions to the brain tissue (p. 458).

Affixing Skin Grafts. *First Choice—Fibrinogen and Thrombin.* When a solution of fibrinogen is applied to one surface and a solution of thrombin to the other, brief firm pressure results in a uniform close adherence of the graft by means of a natural medium which promotes the growth of permanent tissue union and absorbs completely without reaction (p. 448).

Second Choice—Plasma, Fresh Liquid, Frozen, or Dried. These products can be used satisfactorily with either animal or human thrombin. At present, bovine thrombin is generally available while human thrombin and fibrinogen are not.

Hemostasis. Local hemostasis has been simplified and improved by the use of human blood derivatives and substitutes.

First Choice—Fibrin, or Gelatin Foam or Soluble Cotton, Preferably Saturated with Thrombin. These substances can be cut and molded to meet individual requirements and, when applied to small bleeding points, will produce instant hemostasis. Saturation of the

foam or cotton with thrombin, although not essential, enhances the results obtained. These products are absorbed completely without local reaction and have been found especially useful in neurosurgery (pp. 448 and 458).

Second Choice—Fibrinogen and Thrombin Solutions or Powder. These substances also produce rapid hemostasis at small bleeding points. Their use is less satisfactory, however, because there is no preformed matrix to aid in clot formation.

Wound Dressings. The topical application of a protein-rich substance materially enhances wound healing and is of particular value in chronic lesions, such as indolent ulcers, or severe burns (p. 440).

First Choice—Red Cell Paste, Powder, or Ointment. All three of these products give satisfactory results; the cells are usually available as a waste product of a blood bank, and the paste, powder or ointment lends itself readily to topical use.

Second Choice—Plasma, Liquid, Frozen or Dried. Although the results are comparable to those with red cells, plasma is less economical and less satisfactory to use.

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CHAPTER 3

Clinical Aspects of Shock

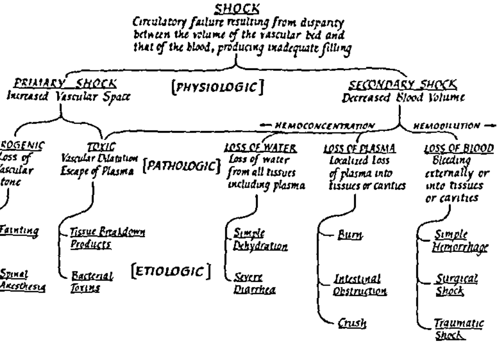
By Robert C. Hardin

The clinical manifestations and etiology of shock vary remarkably. The advancement of a multiplicity of theories concerning its causation and fundamental nature bears witness to this fact and leads to the conclusion that this condition is not completely understood. For over a century workers have searched for a common factor in all cases of shock. In recent years war has twice given impetus and opportunity for such studies and yet there is no complete explanation. It does not suit my purpose to discuss here the several theories concerning shock nor even to examine their historical background. The reader is referred to the excellent review of Harkins[1] for this purpose. It is rather my object to set forth some workable hypothesis for the physician since all are faced periodically with the problem of treating shock. To cope with this emergency the doctor must have a concept of the pathologic physiology of shock, a workable clinical classification, a knowledge of its complications, and, of course, a sensible therapeutic plan.

DEFINITION

There has accumulated in the consideration of shock a somewhat loose nomenclature with many terms which, if not synonymous, overlap in meaning, e.g., *primary* and *neurogenic*. For clarity it is necessary to limit their application. It is impossible to contain a complete definition in a single sentence; only a rather detailed description can be satisfactory. However, at least as a starting

point, shock may be regarded as a state characterized by peripheral circulatory failure caused by a disparity between the volume of the vascular bed and that of the blood, of such nature that the circulatory system is inadequately filled. This viewpoint has been advanced by Blalock in his many writings.[2,3,4] He has further classified shock on the basis of etiology into three types, *hematogenic*, *neurogenic* and *vasogenic*. The first is characterized by loss of blood volume, the second by reflex loss of vasomotor tone, and the last by dilatation of blood vessels due to direct action of toxins on their walls. Two mechanisms, then, may produce shock: increase of intravascular space, or loss of circulating blood volume. The former is *primary* and the latter *secondary* shock. Certain objections have been raised to the employment of these terms[1] but their usage has become universal and cannot be lightly discarded. The first is more common and is often seen alone, but it is doubtful if secondary shock ever occurs uncomplicated at some time in some degree by primary shock. The accompanying diagram serves to depict the various types of shock and some of their interrelationships.



The diagram shows only certain of the more common clinical entities and might be extended almost indefinitely to include many special cases. Neither is it possible to depict all the complicated relationships of the various types of shock. In the average case more than one factor is operating. For example, in hemorrhagic shock complicating surgical procedures there is often dehydration as well as some degree of primary shock. In addition, it is

important to note that primary shock, particularly the toxic type, may rapidly change to secondary shock through increased capillary permeability and loss of blood volume.

PATHOLOGIC PHYSIOLOGY

Primary Shock. The purest example of primary shock is the common phenomenon of fainting. Physiologically it is characterized by a loss of vasomotor tone and a reflex slowing of the heart, having its inception in painful physical or unpleasant psychogenic stimuli. Ordinarily the state of shock is of short duration although it may persist well past the usual few minutes.[5] Recovery is spontaneous and results from the return to normal function of sympathetic control of the circulation. Spinal anesthesia, by interruption of the essential regulatory reflex arcs, produces the same effect.[6] The toxic types of primary shock result from a similar mechanism. Histamine shock is not a clinical entity but demonstrates the essential physiologic events. By direct action on arterioles and capillaries it produces a dilatation with a profound drop in blood pressure. This dilatation results in an increased capillary permeability with loss of plasma from the circulation.[6] Because of this latter phenomenon histamine shock has usually been regarded as an example of the secondary class. It seems more logical, however, because the vasodilatation both precedes and causes the plasma loss, to regard histamine shock as primary in its first stages and secondary in its later development. Several tissue-breakdown products are capable of producing the same changes and these have been for years the subject of much investigation.[7,8,9]

Several substances (histamine, polypeptides, adenosine triphosphate) have been advanced as the causative agent. However, it is apparent that such histamine-like substances are not solely responsible for the production of shock[10,11,12] since careful experimentation has shown shock to be present in many instances in which a toxin could not be demonstrated. In the face of presented evidence, however, one cannot assume that these agents never operate as either the initiating factor or a complicating one. This has been forcibly demonstrated by recent investigators[13] who showed that certain bacterial toxins may produce profound shock, primary or secondary.

Essential Changes in Secondary Shock. In secondary shock the initiating mechanism is unequivocally the depletion of blood volume[14,15,16,17,18,19] which may be due to loss of whole blood, plasma, water and electrolytes, or any combination of the three. Attempts have been made to dissociate hemorrhage and shock but

this is not logical in that diminution of blood volume by loss of whole blood will result in the same physiologic disturbances as loss of any constituent part.[8] Up to a certain point, at least, the physiologic effects of lowered blood volume are well understood. Circulatory failure results from diminished cardiac output which is the direct result of inadequate venous return to the heart.[20,21,22,23,24] This relationship, however, may not be lineal in that central (atrial) venous pressure may fall markedly before cardiac output is decreased.[23] However, as venous return to the heart is progressively diminished, cardiac output falls and peripheral arterial pressure is decreased in spite of the compensatory vasoconstriction. Howarth and Sharpey-Schafer[22] have further shown that although right auricular pressure falls and cardiac output decreases, the arterial pressure is maintained up to a certain point when it falls suddenly. They believe, as do Stead and his co-workers,[23] that this sudden fall may be due to suddenly decreased peripheral resistance.

Other physiologic changes in the heart have been demonstrated which may be directly attributed to the poor venous return.[24,25] These include slow ventricular filling, initial tension decrease in both ventricles, a smaller ventricular diastolic size, and a shortening of the systolic ejection period. Correlation with certain clinical data shows clearly that there is a phase following blood volume loss in which arterial pressure is maintained. During this period clinicians regard the circulatory state as abnormal but compensated. There is agreement that when blood volume is reduced by approximately 30 per cent to 40 per cent compensation breaks down and that the circulation degenerates rapidly.[16,19,17,18]

These are the essential disturbances in secondary shock, and other changes in the circulation or in the organism may be regarded as secondary. Most of these changes have been known for a considerable time. In fact various workers in their search for a causation of shock have regarded one or another of them as the responsible mechanism. As a result of more recent studies, particularly with more refined techniques, it is now possible to view them in proper perspective. Again there has been some confusion concerning definition of terms. By some shock has been regarded as an irreversible state culminating in death. Others, particularly clinicians, use the term shock to indicate the hypotensive stage following loss of blood volume, recognizing that after a variable period of time the organism becomes so damaged that restoration of blood volume does not reverse the process and death supervenes. In the following discussion this latter designation will be followed and the term *shock* will be used in the clinical sense referring to the hypotensive

reversible stage and *irreversible shock* will be used to indicate that stage not amenable to treatment.

Redistribution of Cells. Among the earliest observations in shock was that there is a redistribution of the blood cells of such nature that the capillary bed contains a higher concentration of cells than does the rest of the vascular system.[7,16] This capillary hemoconcentration was thought to be due to local escape of plasma through vessel walls. In fact this latter phenomenon has been used to explain the production of irreversible shock. However, Fine and Seligman[26] have produced evidence that capillary leakage does not occur early enough to account for such local hemoconcentration. At a later date in the same laboratory[27] extensive studies showed that the trapping of erythrocytes in small blood vessels of all organs is an important factor in the reduction of circulating blood volume and impairment of capillary flow. It was further noted that the degree of trapping paralleled the drop in mean arterial pressure but showed no relation to reduced blood volume. Robertson and Bock[16] noted that the discrepancy between the hemoglobin content of blood obtained by ear puncture and that from a vein disappeared when the blood pressure was raised by infusion. It seems apparent, then, that as blood pressure falls, there is concurrently a further deterioration in the circulation brought about by trapping of red cells in capillary beds.

Anoxia. Another well-known observation in shock is that the oxygen saturation of venous blood falls markedly. The relationship of this anoxemia to the production and perpetuation of shock has been the subject of much controversy. Resultant tissue anoxia has been advanced as both an initiating factor and a perpetuating one. It has also been a popular explanation for the state of irreversibility. References to work in this phase of shock are too numerous to mention and the reader is again referred to Harkin's review.[1] The work of Frank and Fine,[28] however, demonstrates that the achievement of normal oxygen tension in arterial and venous blood will neither prevent nor cure shock. They point out that the maintenance of normal oxygen content in the blood in larger vessels does not necessarily lead to proper oxygenation of tissues, since such blood may by-pass capillary beds by arteriovenous shunt, or may fail to enter stagnant areas. Their work indicates that anoxemia is not an initiating factor. It may play an important role in the production of irreversible shock. Nevertheless it must be regarded as an effect and not a cause of circulatory failure.

Lowered Metabolism. In conjunction with anoxemia there is also a lowered metabolism in shock[7] with a very significantly reduced total oxygen consumption and a lowering of body tem-

perature. This apparently follows the reduction in cardiac output and the resultant hypotension. Interestingly enough, the total oxygen consumption may be maintained at normal levels in dogs by the administration of oxygen under pressure without affecting the course of shock from hemorrhage.[28] Again, lowered metabolism would seem to be an effect of shock.

Acidosis. The fourth effect of shock on the organism is the production of acidosis evidenced by a lowered alkali reserve and a reduction of the plasma pH.[7] This develops slowly and closely parallels the drop in systolic blood pressure. It is thought to result from the accumulation of nonvolatile organic acids arising from faulty tissue metabolism incident to anoxia. Maintenance of normal total oxygen consumption and of normal values in arterial and venous blood do not prevent the development of acidosis.[28] This is perhaps evidence that such procedures do not affect capillary beds and that this phenomenon is again the result of circulatory failure.

REMOTE EFFECTS OF SHOCK

The changes just mentioned are a part of the clinical picture of shock. There are others which do not so clearly affect the entire organism. These are changes that take place within a given organ. Little can be noted in many instances insofar as structural changes are concerned but there is considerable evidence of functional impairment. Such changes are widespread and there is reason to suspect that no organ escapes the effects of an inadequate circulation.

Renal Damage. Experienced clinicians recognize the fact that kidney damage is an all too frequent complication of shock. Hemoglobinuric nephrosis (Chap. 12) occurs in a wide variety of conditions in which there is tissue destruction, intravascular hemolysis, or intoxication with drugs or poisons.[29] Since these are often associated with shock, it is difficult to ascertain which may be the primary factor in bringing about renal failure. In addition there is evidence that shock itself may be a causative agent.[29,30,34] Certainly profound alterations incident to shock have been demonstrated in the renal circulation,[31,32,33,35] consisting of a reduced volume of blood flow and increased arterial resistance within the kidney. These result in diminished urinary excretion and markedly reduced plasma clearance. Early treatment of the shock will reverse these phenomena, but a few patients will continue to exhibit oliguria or anuria, hypertension, and azotemia. Death results from uremia. Whether this follows prolonged renal anoxia or whether other agents (myoglobin, hemoglobin, toxins) are primarily responsible

is unknown. It has been suggested[30] that perhaps the damage to the kidney represents the additive effect of shock and excretion of heme pigments. The problem remains to be elucidated but there is no doubt that the possibility of kidney failure is an important consideration in patients suffering from shock.

Cardiac Damage. Cardiac failure also occurs during or after shock much more frequently than one would expect. Electrocardiographic studies have revealed minor and infrequent changes in the function of the conduction system but little else.[36] From animal experiments, however, it is apparent that prolonged hypotension may produce myocardial depression.[25] The relative ease with which the circulation of the shocked patient may be overloaded is perhaps explained in this manner. It may also be the mechanism of death in some cases.

Central Nervous System Damage. Injury to the central nervous system incident to shock is a well-known phenomenon. The brain is often the first organ to fail from anoxia.[1,37] Local oxygen lack for even short periods has an extremely detrimental effect and may result in sudden respiratory failure.

Other Complications. Other less well-understood complications are seen. Richards reported pulmonary edema occurring frequently in the first week following the shock. Whether this results from primary pulmonary damage is not clear.[37] Profound changes take place in hepatic function and in the chemical composition of the liver.[38] Animal experiments have shown changes in carbohydrate metabolism, some of which may be correlated with liver damage. The blood sugar falls and carbohydrate stores are depleted. There is failure of storage of glycogen in both muscles and liver. The administration of dextrose results in high and prolonged levels of the sugar in the blood. Insulin lowers the blood sugar but does not induce the storage of glycogen.[39] There is also depression of dextrose absorption from the gastrointestinal tract. The absorption of water and sodium chloride is not affected, a fact useful in treatment.[40] The effect of shock on the adrenal gland and vice versa has been the subject of considerable study and speculation. Harkins concluded in 1943[1] that the role of the adrenal cortex, even though its functions were known to be depressed, could not be defined. The situation remains much the same but, in the light of other research, elucidation of the problem seems unessential to the explanation of shock. Hyperpotassemia is, however, an interesting phenomenon in shock.[41] It may arise from inactivity of the adrenals but is more probably associated with a shift of fluid from the intracellular space[42] and is reversed by the administration of blood or saline solution.[43] A similar occurrence has been observed in

simple dehydration. Hyperpotassemia may be regarded as evidence of the far-reaching effects of a depleted circulation on the tissues.

IRREVERSIBLE SHOCK

At some point in its clinical course shock becomes irreversible. When that stage is reached, even though the circulation is carefully returned to normal by appropriate therapy, deterioration of the organism continues and death results. There is no agreement among experimentalists as to what the exact explanation of irreversibility may be. For the most part it is agreed that there is loss of plasma from damaged capillary beds which further depletes the circulation.[1,49] However it must be noted that this phenomenon does not occur early.[28] Tissue anoxia[1,45] and toxins [7,41] have been advanced as causative agents of this plasma leakage. Wiggers[25] has recently suggested that myocardial depression might be the operative factor and has also stated[20] that the possibility of exhaustion of vasomotor centers cannot be disregarded. The mechanism of irreversibility has not been established. That shock produces a profound alteration in the entire organism has been adequately demonstrated. Clinically death is seen to occur from cardiac failure, pulmonary edema, respiratory and renal failure, as well as circulatory collapse. It seems logical to assume that there is no one mechanism of death in irreversible shock but that it varies with circumstance and the individual. The patient who dies of uremia a week after being in shock should be regarded as having had an irreversible state just as much as the one whose circulation cannot be maintained at normal. The only established fact concerning irreversible shock is that it requires time to develop. Wiggers[20] has regularly produced irreversible shock in two hours and fifteen minutes by bleeding dogs until the systolic pressure is extremely low (50 mm. of mercury for ninety min. and 30 mm. for forty-five min.). This represents shock of a very severe degree. In man irreversibility develops in from six to eight hours, depending to some extent on other injury and on the degree of oligemia.

COMPENSATORY MECHANISMS

Vasoconstriction. In the discussion so far, the emphasis has been on the progressive deteriorative changes encountered in secondary shock and only passing mention has been made of the compensatory mechanisms. Brief examination of these will add to the understanding of the problem. Foremost of the responses to the loss of blood volume is vasoconstriction. In the past this phenomenon has been

thought to be the causative agent in shock.[20] Later studies[21,46,37,23] have shown it to occur after reduction in blood volume and have assigned to it the role of a compensatory mechanism. Early in shock there may be reflex vasodilatation,[23] i.e., primary shock, but as blood volume is depleted vasoconstriction occurs. Furthermore this response is selective,[37] not all vascular beds being affected in the same degree. This may result in dangerously diminished blood flow to some vital organ, such as the kidney, and has the nature of a two-edged sword. Accompanying vasoconstriction tachycardia develops, which however is of little value in the maintenance of cardiac output or blood pressure.[22] As shock increases in severity these protective phenomena fail either gradually or suddenly. This deterioration may be accelerated by slight trauma or by some minor physical disturbance.[37] It is interesting to note that in some cases overcompensation is seen, [47,48,49,50] evidenced by hypertension with systolic pressures above 140 mm. of mercury and bradycardia. These are instances in which depletion of blood volume is less than 30 per cent. Lower values are accompanied by breakdown of the compensatory mechanisms.

Hemodilution. A much slower corrective phenomenon is the restoration of blood volume by movement of water from other tissues into the blood. This operates only in hemorrhagic shock and is either nonoperative or masked in other types. The mechanism is not only slow but is definitely limited by the available water and the total hemoglobin content of the blood.[16] The administration of fluids by way of the alimentary tract enhances such recovery but must be maintained over several days. Even when large amounts of water are thus available the blood does not dilute when the hemoglobin level is less than 20 per cent. This correlates well with the finding of low blood volumes in patients with severe anemia[51,52] and the proved greater efficiency of whole blood than plasma or albumin solution in the restoration of blood volume after hemorrhage.[37] In the severely shocked individual hemodilatation cannot be counted on to produce any great effect in the first few important hours. In their study of wounded men Ebert and Emerson[19] found an average increase in blood volume of 200 ml. resulting in this fashion.

MECHANISMS OF BLOOD VOLUME DEPLETION

The results of blood volume depletion, except for hemodilution, are the same regardless of its exact character. The variation encountered clinically is not of shock itself but of the process responsible for its production. The diagram on page 25 indicates that

depletion may result from loss of water, plasma, or whole blood. Although lack of one fluid usually predominates, it is unusual not to find a combined diminution of two or three. This colors the clinical picture in varying degree. In addition the underlying disease or injury may produce other changes so that each case becomes an entity requiring special consideration. Failure to recognize this principle leads to the empirical application of remedial measures which, in many instances, will result in therapeutic failure.

Water Loss. Shock from dehydration alone is encountered so infrequently that it is largely of theoretical interest. Some understanding of its characteristics, however, will aid in the treatment of shock due to loss of plasma or whole blood because in such cases there is usually some degree of dehydration. Minor fluctuations of total body water involve only the fluid in the extracellular space. Larger changes affect the intracellular compartment, but reduction of plasma volume does not occur until water loss becomes extreme or unless it takes place rapidly. Clinicially, dehydration from simple water deprivation is rarely seen, the usual cause being vomiting, diarrhea, or excessive sweating. In any instance the deficit is never of the water alone, but is combined with loss of electrolytes. Acid and base ions may be lost in equal amounts as in excessive perspiration, or one may predominate. For example, acid-ion depletion is characteristic of vomiting due to pyloric obstruction, and total base is reduced in diarrhea. Dehydration may consequently be complicated by acidosis or alkalosis. The reader who wishes to pursue this further is referred to Gamble's monograph.[44] In extreme dehydration or rapid water loss diminution of blood volume of sufficient degree may occur to produce shock. This is more likely to occur during the course of some acute infectious diseases such as cholera, or in small children, particularly those with gastrointestinal disease.

Plasma Loss. Plasma loss occurs when there is sufficient change in the vascular walls to allow its escape when there is no interruption of vessel continuity resulting in hemorrhage. A common clinical example is the shock produced by burn.[54,55] The diminution of blood volume is entirely accounted for by local escape of plasma into the damaged tissue and from the surface of the burn. Although plasma loss is the primary factor it is well known that burned patients rapidly develop secondary anemia. It is not generally appreciated that this occurs early and that within the first twenty-four hours an erythrocyte deficit of from 500 to 1000 ml. may develop.[17] Other clinical entities of which loss of plasma is a part include crush injury[56] in which vascular damage is induced by

obstruction of blood flow for a critical period. Upon resumption of circulation plasma escapes from the damaged vessels. It is the clinical counterpart of the experimentally produced tourniquet shock.[57] This type of tissue destruction is particularly apt to lead to renal damage (Chap. 12). Intestinal injury or obstruction, particularly if leading to local circulatory obstruction, may result in plasma loss sufficient to produce shock.[1,15,58] This may occur in volvulus, strangulated hernia, intussusception, mesenteric thrombosis, externally strangulated colostomy loop, and abdominal trauma. It is not apt to accompany intestinal obstruction due to intrinsic tumor.[59] In these cases the plasma diffuses into both the peritoneal cavity and the bowel lumen. In obstruction of the gastrointestinal tract, vomiting with attendant loss of electrolytes may complicate the picture. Harkins[1] adds several clinical entities to this list, including freezing, bile peritonitis, acute pancreatitis, tissue autolysis *in vivo*, pneumonia, and pulmonary edema. Hemorrhage may be a complicating factor in many of the cases, characteristically producing plasma loss. For example, in crush injury there is often considerable extravasation of blood into the tissues of the involved extremity and in abdominal injury frank bleeding is a frequent accompaniment.

Blood Loss. Perhaps the most commonly encountered mechanism responsible for reduction of blood volume is hemorrhage. Bleeding occurs when there is a break in the continuity of blood vessels. The loss may be external or into body cavities, hollow organs, tissue planes, or into the tissues themselves. Examples of each are easily enumerated but severed arteries, ruptured spleen, bleeding peptic ulcer, and compound fracture may be mentioned.

Magnitude of Blood Loss. Hemorrhagic shock results from loss of startlingly large amounts of blood. A blood donor submits to the removal of 500 to 600 ml. without ill effect. Studies[60,61] have shown losses of 700 to 1200 ml. to be tolerated by the normal subject. Blalock[3] and others[15] have pointed out the enormous capacity of the human organism to compensate for the ill effects of hemorrhage. However, this is only a matter of degree, for shock ensues from hemorrhage in excess of 30 per cent of the total blood volume.[17,19,61] This requires in the average individual a loss in excess of 1500 ml. Although it is easy to visualize blood loss of such magnitude from a break in the continuity of a large artery, it may be somewhat surprising that shock resulting from surgical procedures or following trauma is due to hemorrhage.[37,19,17,18,62] War wounds leading to shock are characterized by an average depletion of blood volume by hemorrhage, varying with the anatomical location of the wound, of 70 per cent in extremity and perforating wounds of the chest or

abdomen combined, 60 per cent in extremity wounds alone, 35 per cent in chest wounds, 40 per cent in abdominal wounds and 50 per cent in chest and abdominal wounds combined.[19] The degree of whole blood loss commonly encountered in hemorrhagic shock in civilian practice does not differ significantly.[18,62] Gastrointestinal hemorrhage, head injuries, abdominal injuries, and fractures have all been reported to produce bleeding of this degree. Postpartum hemorrhage producing shock is recognized as one of the more common complications of delivery.[63] That surgical procedures are attended by considerable hemorrhage has been demonstrated by measurement of shed blood and by determination of blood volume before and after operation (Table II).[64,65,66,67,68,72,19,17] The amount of blood loss varies with the anesthetic and the nature of the operation. Amounts have been recorded ranging from 25 ml. in a simple appendectomy to 3200 ml. in the amputation of an arm combined with thoracotomy. The conclusion is inescapable that shock during surgery is primarily due to hemorrhage.

CLINICAL DIAGNOSIS AND CLASSIFICATION

General Considerations. Depletion of blood volume by loss of whole blood, plasma, or water is encountered in many disease processes. Clinically it is most commonly seen in gastrointestinal hemorrhage, trauma, and during surgical procedures, where whole blood is lost, and in burn or intestinal lesions where plasma is depleted. Emphasis cannot be too great on the fact that secondary shock is an integral part of so many clinical entities. It is upon this realization that early diagnosis and proper treatment are based. A knowledge is required of when shock may be encountered and how much loss of blood volume may be expected under given conditions. The treatment of shock is in reality always preventive. Thus, if it is known that a certain surgical procedure is likely to be attended by shock, the blood volume may be maintained by proper therapy and the results of its depletion circumvented. In a patient suffering from trauma whose compensatory mechanisms have maintained an adequate circulation but who is on the brink of circulatory failure, the proper administration of blood may prevent deterioration. Likewise the patient in the hypotensive stage may be saved the organic damage of an irreversible nature. Successful treatment requires a thorough understanding of the clinical possibilities and an ability not only to recognize blood volume depletion but also to assess its nature and severity. This may be logically accomplished by seeking the answers to three questions: (1) Is the blood volume depleted? (2) What blood component or

components are missing? (3) What is the amount of the component missing?

The appearance of the patient with secondary shock needs little emphasis. Pallor, shallow rapid respirations, tachycardia, apathy or apprehension, cold skin, collapsed veins, and thready pulse make up the usual picture. *None of these, however, nor any combination, is pathognomonic of shock.* Considerable reliance may be placed upon the demonstration of hypotension (systolic blood pressure less than 90 mm. of mercury), for it is the most constant clinical sign of shock.[19,18,5,68] The most important consideration remains, however, the recognition of a clinical state in which blood volume depletion may be expected.

Demonstration of Blood-Volume Depletion. Determination of the presence of blood volume diminution is actually the basis of diagnosis of secondary shock. Its recognition would be greatly simplified by some easily applicable method of exact measurement. The use of the dye T 1824 is relatively simple (p. 222 but even this requires equipment, personnel and time which preclude its universal application. Methods of calculating blood volume after the infusion of plasma (p. 219) have the same failing. Wangensteen has described a simple method of determining the amount of blood loss during surgical procedures[67] by weighing sponges before and after operation.

For the most part the clinician must depend upon his judgment and experience to make the diagnosis. Discovery of hemorrhage by simple inspection or obtaining a history of bleeding may solve the problem quickly. In those cases where the loss is not so evident a thorough knowledge of what types of disease or injury are most likely to be accompanied by blood-volume depletion is indispensable. To the physician who possesses such clinical awareness other signs and symptoms have greater significance.

Nature of the Blood-Volume Depletion. The next step is to determine what part of the blood has been lost. Again the underlying disease process proves the most ready indicator. An understanding of the consequences of various pathological entities enables the physician to identify the particular deficiency with great accuracy. In most instances, as has been previously stated, there is loss of water, plasma, and whole blood, but one predominates. Certain laboratory procedures are useful in further defining the nature of the loss. Of these the hematocrit is the most easily measured and offers particularly valuable information. In hemorrhagic shock hemodilution occurs, and hemoconcentration results from plasma loss or dehydration. Hemodilution following hemorrhage is a relatively slow process so that in the precious

few hours when treatment may be successful the fall in the hematocrit reading may not be marked.[37,49] The degree of variance from normal depends not only on time but will reflect also the state of the blood before hemorrhage. In fact, the finding of a markedly reduced hematocrit shortly after hemorrhage is indicative of a preexisting anemia.[62] The significant change in the hematocrit is an elevation which results from loss of plasma or water. Direct measurement of plasma and cell volumes by methods previously mentioned will, of course, answer the question unequivocally. However, the clinical picture and a hematocrit determination will usually suffice to determine the predominant component lost from the blood.

Degree of Blood-Volume Depletion. The final question to be answered is the extent of the blood-volume diminution. This is most logically presented by considering each type of depletion separately.

Water Loss. Shock due to uncomplicated water loss occurs only in cases of extreme alteration of fluid balance. Before clinical signs of dehydration appear the water loss must be in the neighborhood of 6 per cent of the body weight, about 3600 ml. in the average adult.[72] Although the blood participates to some extent in this loss, the amount is not sufficient to elevate the hematocrit greatly and the blood volume is not significantly altered. Further water depletion diminishes the blood volume, provided that it occurs rapidly enough to prevent action of the mechanisms normally protecting the plasma volume. When a state of anhydremia is thus developed, hypotension and marked hemoconcentration appear. Because the water content of the blood is so well protected it is impossible to correlate the appearance of shock with the percentage loss of total body water, but it may range between 10 and 20 liters. As in plasma loss (q.v.) the hematocrit may be used to determine the deficiency in the blood itself but the value does not reflect, except indirectly and inaccurately, the loss from the intracellular or interstitial compartments.

Plasma Loss. Estimation of the degree of blood-volume depletion, when the loss has been of plasma, may be easily made except in burns after the development of secondary anemia. For this purpose the hematocrit determination is admirably suited. An elevation of one in the reading (above the normal of 45) *corresponds to the loss* of 100 ml. of plasma.[69] Hypotension appears when the blood volume has been depleted by approximately 35 per cent.[18] This may be calculated to correspond with a hematocrit of 60 to 65. This compares well with animal experiments in which the blood pressure has been correlated with hematocrit readings.[70] Harkins[70]

also quoted two other formulae for calculation of plasma deficit in burns.

(*a.*) *Method of Black*

$$\text{ml. of plasma deficit} = \left(5 - \frac{500}{Hb_2}\right) 1000$$

where Hb_2 equals the observed hemoglobin expressed in per cent of normal.

(*b.*) *Method of Elkinton, Wolff, and Lee*

$$\text{gm. of plasma protein deficit} = 3.5 - W \frac{W\,(100-H_o)\,H_n\,P_o}{2\,(100-H_n\,H_o)}$$

where W = body weight in kg.
H_o = observed hematocrit
H_n = normal hematocrit
P_o = observed plasma protein in gm. /100 ml.

These methods may be applied to cases of plasma loss from other causes. Harkins also stated a first-aid formula for estimating plasma deficit from the percentage of body surface involved by deep burn. Fifty milliliters of plasma loss is assumed for each 1 per cent of the body surface so involved. Berkow's method for estimating the extent of a burned area is used (Table I).[71]

TABLE I

Surface Area Proportions (after Berkow)

Part of Body	Per Cent Body Area
Head	6
Trunk	38
Anterior Surface	20
Posterior Surface	18
Upper Extremity	18
Arms and Forearms	13 5
Hands	4 5
Lower Extremity	38
Thighs	19
Legs	12.5
Feet	6.5

Blood Loss. When blood volume is diminished by the loss of whole blood the hematocrit is of no value in estimating the amount of hemorrhage. Other easily elicited signs must be depended upon. Of these the blood pressure is the most reliable.[19,18,5,68] The pulse rate cannot be taken as an indicator of the degree of blood loss because it varies independently of this factor.[47] In their study on wound shock Ebert and Emerson[19] demonstrated that a systolic blood pressure of less than 85 mm. of mercury was indicative of a blood volume loss in excess of 25 per cent (1500 ml. in the average man). Inversely, a systolic blood pressure of more than 100 mm. is evidence that the loss is less than 25 per cent. Similar findings

have been reported in other types of traumatic shock.[19] Furthermore a direct relationship exists between the fall in systolic pressure and the reduction of blood volume. The loss of 500 ml. in blood volume may be expected to be reflected in a drop of from 10 to 20 mm. of mercury in the blood pressure.[5] An exception to this is found in injury of the central nervous system. The phenomenon of overcompensation by vasoconstriction may mask the blood volume loss for a time. Another useful clinical indication of the amount of blood loss in traumatic cases is to be found in the nature and extent of the injury. Reference to page 40 will recall the amount lost in various types of trauma. Taking blood loss as the criterion, injuries may be classified into three groups: thoracic, abdominal, and skeletal. Of these, the first is characterized by the least bleeding and the last by the greatest. It is interesting to note that in instances of multiple fractures the amount of hemorrhage is directly proportional to the number of fractures.[43] Similarly a certain amount of hemorrhage is apparently an inherent part of surgical procedures (Table II). Thus blood loss may be roughly estimated from the nature of the underlying disease process or its surgical treatment.

TREATMENT

Once the three questions of whether the blood volume has been depleted, the constituent of blood lost, and the amount, have been answered, the treatment becomes obvious. Qualitative and quantitative replacement and thus restoration of the circulation is the prime objective. Transfusion may be regarded as a tissue graft and because of the availability of whole blood, plasma and its byproducts, and crystalloids, replacement therapy may be nicely adjusted to the need of the individual case. When one considers the pathologic physiology of shock, however, it becomes evident that whole blood, plasma, and isotonic saline solution will make up the bulk of material used for replacement.

Replacement of Water. The treatment of shock encountered in dehydration is essentially correction of the underlying state. Dehydration may vary in character according to its cause, being complicated in some instances by excessive loss of either acid or basic ions. In simple water deprivation, in loss of water combined with base (e.g., diarrhea), or in loss of water combined with acid (e.g., vomiting in pyloric obstruction) the fluid of choice is isotonic (0.9 per cent) sodium chloride solution.[44] Given normally functioning kidneys, the organism will selectively excrete the unneeded ion and retain the other, together with the water. The volume necessary for correction of dehydration may be estimated by

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a.) *Method of Black*

$$\text{ml. of plasma deficit} = \left(5 - \frac{500}{Hb_2}\right) 1000$$

ere Hb_2 equals the observed hemoglobin expressed in per cent of normal

b) *Method of Elkinton, Wolff, and Lee*

$$\text{gm of plasma protein deficit} = 3.5 - W \frac{W\,(100 - H_o)\,H_n\,P_o}{2\,(100 - H_n\,H_o)}$$

ere W = body weight in kg
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The administration of large volumes of fluid by any route requires hours and one has the clinical improvement of the patient to serve as a further guide. Because of this time factor, other considerations must enter into the calculation of amount of fluid to be administered. These are the daily requirements of water for the maintenance of urinary excretion and temperature control. Coller and Maddock[71] have shown that the daily needs for these purposes include 1000 to 1500 ml. for urinary excretion and 1500 ml. for insensible perspiration and pulmonary evaporation. In fever approximately 500 ml. for each degree of Fahrenheit temperature elevation must be added. These additional amounts cannot be disregarded in treatment extending over a period of hours.

Dehydration shock is not commonly encountered but the subject of its treatment remains important because water loss of greater or lesser degree is a concomitant of other types of shock. The sudden depletion of plasma or whole blood volume, even by large amounts, does not produce a marked degree of dehydration because the mass of body water is not appreciably affected. The degree of dehydration in such cases bears no relationship to the severity of the shock but is more dependent upon other circumstances. The state of water balance at the time of development of shock is the determining factor. A warm environment, limitation of fluid intake, or the underlying disease may have produced an unfavorable balance of clinical significance.

Methods of Administration of Water. Replacement of water is a necessary component of the treatment of shock. Every method of administration is effective and the choice of technique will be dictated by clinical circumstance. Perhaps the most neglected and at the same time in some ways the most effective route is by the alimentary tract, either orally or rectally. Administration of fluid by mouth should be instituted as soon as feasible. It matters little in what form the water is given so long as alcohol, which is detrimental in shock,[37] is not included. Warm fluids are well tolerated but hot drinks are to be avoided. The giving of isotonic saline solution by proctoclysis is clinically proved efficacious, not only in combating dehydration but in producing permanent increase of blood volume. The alimentary administration of water results in augmentation of the plasma volume by the increment of protein-containing fluid. Over a period of one or two days increases of 600 to 1100 ml. have been observed.[16] This is too slow to be relied upon as the sole method in routine treatment. It is of great value, however, in combination with other replacement therapy. Isotonic saline solution by the intravenous route is useful in the treatment

TABLE II

Blood Loss During Operations (in ml)

Types of Operations	Investigators						
	Baronofsky *et al.*[87]	Emerson and Ebert[19]	Gatch and Little[63]	Coller *et al.*[73]	White *et al*[64]	White and Buxton[66]	Nesbit and Conger[65]
Abdominal Operations		2200					
Appendectomy	25 8		8				
Cholecystectomy	179 4						
Biliary Tract				594			
Resection of Colon	121 5						
Gastric Resection, Ulcer	499 8			321			
Gastric Resection, Cancer	455 6						
Gastrectomy, Total				804			
Abdominal—Perineal				410			
Thoracic Operations							
Thoracotomy		600					
Pneumonectomy							
Average	1399.0					1458	
Cancer	1534 0						
Bronchiectasis	1324.3						
Thoracoplasty						495–773	
Revision						952–1009	
Tuberculosis Empyema						411–1106	
Lobectomy						1607	
Hernioplasty	82 9						
Mastectomy							
Radical	415 4		710	821			
Simple			410				
Thyroidectomy							
Average	405 6		216	379			
Diffusely Enlarged	668 0						
Nodular	206 0						
Hysterectomy			257				
Nephrectomy			816				
Prostatic Resection							4–1254
Neurosurgical Operations							
Laminectomy					334–1263		
Fracture			672				
Craniotomy							
Simple					500–900		
Avascular Tumor					600–1200		
Large Meningioma					2000		

application of known experimental data. The appearance of clinical signs of this condition indicates a depletion of approximately 6 per cent of body weight. Shock does not result from loss of this degree and hypotension occurs only in excessive or rapid depletion of body water.

protein = 13 to 15 ml. plasma).[74] In the face of marked dehydration concentrated solutions are contraindicated.

Problem of Burns. *Initial Use of Plasma.* In the special instance of burn there is agreement that the initial replacement should be with plasma. The required dosage may be calculated by any of the formulae already given. It must be remembered, however, that in burn the early appearance of secondary anemia is a constant feature. This apparently arises from destruction of erythrocytes and may be accentuated by hemorrhage.[78,79] The resulting deficit, which may be as much as 1000 ml. within twenty-four hours,[17] renders the hematocrit unreliable as a measure of total blood volume loss. This determination retains its usefulness as a qualitative indicator of plasma loss and for calculation of minimal plasma replacement, but a return to normal hematocrit reading by the infusion of plasma does not necessarily result in the restoration of normal blood volume.

Use of Whole Blood. Depending upon the degree of erythrocyte destruction, there is a remaining deficit which is of plasma and red blood cells or, in actuality, of whole blood. The discovery of the size of this deficit and the decision as to when whole blood should be administered are difficult. Animal experiments[80] and clinical studies[81] have shown that transfusion of whole blood is not contraindicated by hemoconcentration. Early use of whole blood is desirable in order to correct the inevitable anemia. Evans and Bigger[81] have shown that such a procedure not only prevents anemia but observed that toxemia was less, plasma protein levels were better maintained and that epithelialization of the burned surface was rapid. In these studies whole blood was used to the exclusion of plasma and the importance of adequate water intake, preferably by mouth but supplemented intravenously, to provide a satisfactory urinary output was stressed. The patients were also given sodium bicarbonate intravenously in a dosage of 8 gm. with each transfusion. This follows the work of Moyer *et al*,[80] who observed that blood transfusion and sodium bicarbonate by mouth were more efficacious than plasma or blood alone in the treatment of shock due to burn. It therefore seems well established that whole blood transfusion is a desirable component of the treatment of shock in burn. Judgment of the volume to be used depends upon the estimation of red cell and plasma volumes. Such information may be gained by application of the method of measurement (p. 227) based on hemoglobin concentration before and after infusion of a known volume of plasma. Such a determination may be made during the initial administration of plasma, and qualitative and quantititative replacement may follow. Loss of blood volume

of dehydration but because, in contradistinction to the alimentary route, it produces a simple dilution of the blood with salt water, little permanent augmentation of the blood volume is achieved. The water and sodium chloride rapidly leave the blood and any increase in volume is, for the most part, extremely transient. The amount of permanent increase has been demonstrated to be less than 10 per cent in hemorrhagic shock.[37] The third route of administration of saline solution is by hypodermoclysis. Large volumes of fluid may be injected safely in this way without the attendant danger of circulatory overload, which is encountered in intravenous administration.

Other Fluids. Although the fluid of choice is usually water or saline solution, under certain circumstances dextrose or potassium chloride solutions may be necessary. When the intravenous route is chosen and the patient is in electrolyte balance or is in need of water for urinary excretion, 5 per cent dextrose in distilled water is indicated.[53] In severe dehydration, when loss of potassium from the body has occurred, the administration of potassium chloride solution by hypodermoclysis is efficacious.[77] The attendant danger of the effect of massive doses of potassium salts on the heart must be guarded against by careful attention to technique with special regard to amount and rate of flow.

Replacement of Plasma. In most cases of clinical shock the use of crystalloid solutions fills a secondary role, and blood or plasma administration is necessary to restore a normal circulation. When plasma has been lost the choice of fluid for transfusion is obvious.

Human serum albumin solution is, within certain limits, a satisfactory substitute in these cases[74] but has no advantage over plasma (p. 13). The amount to be given will be determined by the clinical estimation of the blood volume loss, by measurement of the hematocrit, and by the progress of the patient during therapy. The route of administration is, of course, intravenous. The clinical entities of which plasma loss is characteristic are progressive so that during and after the initial administration careful observation of the patient must be kept. In the event of a rise in the hematocrit value or a drop in blood pressure further infusion may be necessary. Frozen, dried, or liquid plasma are equally valuable and completely interchangeable. Plasma or albumin may be given in the concentrated state with good results insofar as increase in blood volume is concerned and without danger even in the presence of moderate dehydration.[74] However, nothing is gained by such procedure and it is not recommended. Dilution to the isotonic state furnishes needed water and obviates the rather artificial conversion of grams of protein to milliliters of plasma volume (1 gm.

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continues in burns for a period of approximately seventy-two hours,[70] so that the patient must be closely observed. Further administration of plasma or blood must be used to maintain a normal circulation. Determination of the hematocrit, blood volume, blood pressure, and, perhaps most important, urinary flow are useful in estimating the adequacy of the circulation. Evans and Bigger suggest that the output of urine should be 50 to 100 ml. per hour. Such an output is indicative not only of an adequate volume of available water but also of a normal circulation. Part of the replacement in the first twenty-four hours should consist of whole blood and the need of further transfusion will be indicated by those determinations listed above and by the progress of the patient. In view of the recorded success with whole blood alone it seems advisable that, when any doubt exists, blood should be administered in preference to plasma.

Replacement of Whole Blood. In hemorrhagic shock the replacement is best made with whole blood. A certain amount of substitution of plasma or albumin solution may be made but in the more severe cases too free use of these products will be attended by therapeutic failure. Methods of determining the amount of blood to be transfused have been discussed above. In severe shock (i.e., depletion of 25 per cent of the blood volume) the initial dose will be between 1250 ml. and 2500 ml. The progress of the patient will be found useful in measuring the success of therapy. An increase in systolic blood pressure of 10 to 20 mm. of mercury should accompany transfusion of each 500 ml. of blood.[5] However, as in other types of shock, the blood-volume depletion due to hemorrhage tends to be progessive. Therefore the improvement of the patient may not always be proportional to the volume of blood transfused, and a further allowance must be made for bleeding during treatment. This factor is particularly significant in traumatic shock, in which instance as much as 50 per cent of the blood transfused may be so lost.[19] The response of the patient is therefore of prime importance and frequent reevaluation of his status is a necessity.

Substitution of Plasma or Albumin. In many cases in which hemorrhagic shock occurs surgical treatment is indicated. This invariably results in further hemorrhage of greater or lesser degree which must be taken into account. It is evident from these considerations that enormous quantities of blood may be required. This forces one to inquire if some substitute may not be used for at least a portion of the replacement therapy. Experienced clinicians see no objection to the use of plasma, serum, or albumin in considerable quantity and some habitually begin treatment with one of these substances.[65,5,74] However, this practice must be

definitely limited and a substitution of more than 1000 ml. is to be avoided.[19] The use of these substances produces a permanent hemodilution, a secondary anemia, and, if carried to excess, diminishes the oxygen-carrying capacity of the blood to dangerous levels.[17,19] In addition the incompletely understood function of the erythrocyte in the maintenance of blood volume must be considered. Whole blood has been shown to be much more efficacious in producing permanent increase in blood volume than albumin solution[17] or plasma. Low erythrocyte concentration in the circulating blood is incompatible with normal blood volume.[16] The use of protein-containing blood derivatives in hemorrhagic shock is to be regarded as an emergency or supplementary measure. In actuality there is no physiologically satisfactory substitute for whole blood.

Considerations Common to the Use of Plasma and Whole Blood. Certain considerations common to shock from loss of plasma or loss of blood remain to be discussed. Certain techniques of administration are perhaps the most important.

Selection of Vein. Some thought should be given to selection of the particular vein through which blood or plasma is to be administered. The antecubital vein is the usual choice but any superficial vein from the scalp to the foot may be employed. The femoral vein has at times been utilized (p. 253). One important factor in the choice of a vein is that the treatment thus started usually continues for a period of several hours. If the patient's condition demands other attention such as debridement of a burned surface, it is well to select a vein so situated that there will be as little interference as possible by one procedure with the other. In traumatic shock, particularly, one should choose a vein as remote from the injury as possible.

Velocity of Injection. Speed of flow is another important factor. It is determined by the viscosity of the solution, the pressure within the transfusion apparatus, and the caliber of the needle. Only the last two can be varied by the transfusionist. In severe shock the first 500 to 1000 ml. should be given rapidly. A 16- or 18-gauge needle will allow a sufficiently free flow. The first liter should be administered in one half of an hour.[5] The first 500 ml. may be given at a rate two or three times as great (5 minutes). This may be accomplished either by administration under pressure or by simultaneous transfusion into two veins, employing the gravity method and large (16-gauge) needles. After the first liter, the rate of flow is determined by clinical judgment. When the systolic pressure has risen to 100 mm. of mercury the flow may be reduced to 5 to 10 ml. per minute.

Coincident Treatment. It must be remembered that treatment of shock is usually but one phase in the management of the patient. There should be no compartmentalization of therapy with one phase completed before another is begun. In many instances when shock is sufficiently overcome, surgical treatment is in order. The experienced therapist will not discontinue transfusion until the patient has recovered from anesthesia. When the primary objective of replacing lost blood volume has been attained, he will reduce the rate of flow to a bare minimum necessary to prevent coagulation in the needle. As the need arises he can then increase the rate to compensate for blood volume loss during other phases of treatment.

Failure to Respond to Therapy. Unfortunately not all patients in shock respond to replacement therapy. The most common cause of failure is continued loss from the blood stream.[6,19] Another event which unfavorably affects the patient is infection.[19,16,60,13,69] The sudden deterioration of a patient with abdominal injury or intestinal obstruction may be taken as evidence of peritoneal contamination. Gas gangrene is also notorious in aggravating shock. There is also evidence that toxins may be absorbed from badly infected or otherwise damaged tissues which produce the same effect.[60,9,70] Failure to respond to blood volume replacement, or sudden circulatory failure during the course of treatment, should suggest the possibility of immediate surgical intervention to remove damaged tissue or to combat an infection. Finally, failure may be due to the fact that circulatory collapse has been present long enough to produce irreversible damage. No drug or other therapeutic agent has yet been discovered which is of value in irreversible shock.[75]

Complications of Treatment. The treatment of secondary shock is not without inherent danger. Hemolytic transfusion reactions, pyrogenic reactions, and simple overload of the circulation (Chap. 12) are of grave significance in shock. The latter is perhaps the most common complication arising from replacement therapy itself.

Adjunct Measures in Treatment. Several adjunct measures have a time-honored place in the treatment of secondary shock. Although depletion of blood volume is the precipitating cause, several other factors play an important role.

Fatigue and Pain. Fatigue and pain produce what is in reality a superimposed primary shock, or prevent the action of compensatory vasoconstriction. Countermeasures against these are legion, consisting of those simple nursing procedures designed to promote the patient's comfort. The smooth sheet, the properly

adjusted pillow, and the careful placement and support of an injured extremity are but examples. Morphine or similar drugs are invaluable for their pain-relieving and rest-promoting qualities. They must be used judiciously, however, because, when given subcutaneously, absorption may not be complete during the period of circulatory failure. Lack of therapeutic effect may lead to repeated dosage which, when the circulation is restored, may result in the sudden absorption of a toxic amount. This may be avoided by careful attention to total dosage and the time interval of administration.

Application of Heat. One time-honored custom in the treatment of shock may be discarded. This is the swaddling of the patient in many blankets together with hot water bottles or other heat radiating apparatus. These measures are more apt to do harm than good. Animal experiments[76] and clinical experience warn against overheating. Furthermore the production of sweating results in the loss of precious water and electrolytes.[73] The patient should be kept comfortably warm, with blankets if necessary, but care should be exercised to avoid overheating.

The Shock Position. The elevation of the foot of the bed is of some value[37] but should be omitted from the treatment of those patients in respiratory failure or suffering from thoracic or cranial injury.

Immobilization of Fractures. Immobilization of fractures is an extremely important measure. Sandbagging, splinting or casting will help to prevent further extravasation of blood at the fracture site, which may reach serious proportions.[62]

Continued Hemorrhage. In all instances bleeding should be stopped where possible by bandage, tourniquet, ligature, or the application of hemostatic agents. The tourniquet should be used with great precaution against the development of further local circulatory damage.

Oxygen. The administration of oxygen has been a subject of controversy for some time. It does no harm in any case, and when there is pulmonary injury or disease it is of great value. However, the added oxygen content of blood cannot be expected to benefit those tissues which are deprived of blood supply by reason of circulatory failure. Oxygen therapy together with all other measures is simply a supplement to the basic treatment which is replacement of blood volume.

Complications. These have been discussed previously together with the other pathologico-physiologic effects of shock. No further discussion will be undertaken except to recall that cerebral damage, pulmonary edema, cardiac failure, and renal failure may become clinically apparent either during the course of treatment or even

several days after the acute episode. The physician must be ever alert for the appearance of oliguria, shortness of breath, and increased venous pressure. During the period of convalescence the patient's regimen should include a sufficient caloric intake with adequate protein (70 to 100 gm. per day), adequate sodium chloride (5 to 7 gm. per day), and a liberal amount of water. These are preferably administered by mouth.[37] Specifically to be avoided if possible during this stage is the intravenous administration of large quantities of fluid.

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CHAPTER 4

The A-B-O Blood Groups

By Elmer L. DeGowin

It is common knowledge that human bloods can be differentiated into four groups. For the last half century this generalization has proved useful and it will continue to be employed. But there are certain exceptions which occasionally complicate the problems of blood transfusion and these require study in some detail.

THE FOUR CLASSIC BLOOD GROUPS

In 1900 Landsteiner[1] studied the blood of different workers in his laboratory by crossmatching the erythrocytes from each individual with the sera of the others. He found that the serum from some bloods caused the cells of others to clump in a characteristic manner. From these observations he concluded that there were three groups of human blood. In 1902 Decastello and Sturli[2] described the fourth blood group.

Landsteiner postulated that the fundamental characteristics of the four groups are determined by the presence or absence of one or both *isohemagglutinogens A* and *B* in the erythrocytes of the individual. Isohemagglutinogens* are substances in the red cells which combine with specific antibodies in the plasma of human blood to produce clumping or *isohemagglutination*. Whenever the A or B substance is absent from the red cells of a blood, anti-A or anti-B agglutinins develop naturally in the plasma. It follows that agglutinins which act against the A or B agglutinogens never develop in the presence of these agglutinogens, otherwise the

* The prefix *iso-* means *alike* or *from the same species*. When the meaning is clear, the double prefix *isohem-* is frequently omitted for the sake of brevity.

erythrocytes would be clumped and the result would be incompatible with life.

From Landsteiner's concept the blood groups were named for the agglutinogens in the cells according to the following schema:

Landsteiner or International Nomenclature

Name of Blood Group	*Agglutinogens in Cells*	*Agglutinins in Serum*
AB	A and B	none
A	A	anti-B (β)
B	B	anti-A (α)
O*	none	anti-A (α) and anti-B (β)

The relations between the four groups of human blood are shown in Table III.

TABLE III

Interactions of the Four Groups
(+ = agglutination)

Cells	AB Serum (no agglutinins)	B Serum (anti-A)	A Serum (anti-B)	O Serum (anti-A and anti-B)
AB	−	+	+	+
A	−	+	−	+
B	−	−	+	+
O	−	−	−	−

Three facts should be noted from this table: (1) Any unknown blood can be assigned to its proper group by noting the reactions of the erythrocytes to the anti-A and anti-B sera. (2) The cells of group O are not agglutinated by the sera of any other groups and, therefore, with certain limitations (p. 55), this group may be employed as the *universal donor* to transfuse to any other group. (3) The plasma of group AB contains no agglutinins and therefore persons of this group can receive blood transfusions of any other group. This group is sometimes termed the *universal recipient.*

Other Nomenclatures. In 1907 Janský,[3] a Czech, published a paper in an obscure Bohemian journal in which he designated the names of the four blood groups by the Roman numerals I, II, III, and IV. In 1910 Moss,[4] an American, independently named the four blood groups by a similar method, but in a different order. Because of priority the Janský nomenclature was widely adopted in Europe and to some extent in the United States, whereas many Americans clung to the Moss classification. This

* This is the capital letter *O* (so pronounced), standing for Zero.

led to much confusion. As the subject became more complicated it was realized that numerical classifications were not sufficiently descriptive. The Division of Hygiene of the League of Nations recommended the adoption of the Landsteiner letters and this has been widely accepted. It is the only system which permits an adequate comprehension of the interrelationships of the blood groups. The equivalents are:

Equivalent Nomenclatures

International	*Janský*	*Moss*
AB	IV	I
A	II	II
B	III	III
O	I	IV

Isohemagglutinins Anti-A and Anti-B. The anti-A and anti-B agglutinins occur naturally in the plasma of blood in which the corresponding A or B agglutinogens are absent. These antibodies may therefore be termed *natural antibodies*. The titer may be increased by isosensitization, in which case the increment must be regarded as due to *acquired* or *immune antibodies* (Chap. 8). The natural anti-A and anti-B agglutinins have the following characteristic properties: (a) they act maximally at about 5° C., although the thermal range of activity includes 37° C.; (b) they agglutinate erythrocytes suspended in saline solution; (c) they are absorbable; (d) the cells forming the resulting agglutinates adhere very firmly to one another so that hard shaking or dilution of the suspending medium does not disperse the clumps. These properties are discussed in detail on page 120.

Isohemagglutinins, like other antibodies, are found in the globulin fraction of the plasma. They can also be demonstrated in other body fluids which contain plasma globulins, such as lymph, exudates, transudates, and milk. Ordinarily they are not found in tears, saliva, urine, cerebrospinal fluids, or amniotic fluid. However, when globulin appears in the urine in pathologic states the antibodies may also be present.

Isohemolysins Anti-A and Anti-B. When fresh plasma or serum containing natural agglutinins is combined with erythrocytes having the specific agglutinogens, the red cells in the resulting agglutinates may be completely disrupted so that the hemoglobin escapes. This phenomenon is termed *isohemolysis* and is caused by the action of natural antibodies called *isohemolysins*, which have the same specificity as the natural agglutinins in the same plasma. Natural hemolysins occur in about 30 per cent of the fresh plasma or sera containing anti-A or anti-B agglutinins. They never occur

in the absence of the corresponding agglutinins. This type of hemolysis requires the presence of complement in the serum-cell mixture so that it does not occur when the serum has been inactivated by aging or by heating at 56° C. for one hour. The titer of the isohemolysin is usually weaker than that of the corresponding isohemagglutinin. Acquired isohemolysins seem to be extremely rare (p. 96). The presence of isohemolysins may interfere seriously in crossmatching blood for transfusion (Chap. 9).

THE SUBGROUPS A_1, A_2, AND A_3

When serum from group B or O blood, containing the natural agglutinins anti-A, is tested against erythrocytes having the A agglutinogen, about 20 per cent of A bloods and 35 per cent of AB bloods in European stock are found to have notably weak agglutination. The phenomenon is not readily demonstrated with acquired agglutinins. It was shown by von Dungern and Hirschfeld[8] that this difference in response to anti-A serum is due to the presence in the serum of B and O blood of two agglutinins which are now termed *anti-A* and *anti-A_1*. When suitable procedures are employed (p. 148) the addition of weakly-reacting A cells to the serum will absorb the anti-A agglutinins, leaving only anti-A_1 antibodies. Such a serum is frequently termed *absorbed B serum*. When the erythrocytes of group A or AB are not agglutinated by the absorbed serum the blood is designated as *subgroup A_2* or *A_2B*, as the case may be, whereas those agglutinated by the anti-A_1 serum are termed *A_1* or *A_1B*.

Irregular Agglutinin Anti-A_1. Although the anti-A_1 agglutinin occurs regularly in the plasma of group B and O blood, it also occurs naturally in the serum of 1 to 2 per cent of bloods belonging to subgroup A_2 and in 25 to 30 per cent of those classified as A_2B. In these instances, it is convenient to regard the antibody as an *irregular agglutinin*.

Irregular Agglutinin Anti-O (Anti-A_2). This is an antibody which occurs naturally in the serum of about 0.6 per cent of subgroup A_1, in 3 per cent of A_1B, and rarely in group B. It agglutinates the cells in all group O bloods and the erythrocytes in about 95 per cent of those belonging to the subgroup A_2. The bloods of the subgroup A_2 which react are thought to have the genotype *A_2O* and the reaction is due to the presence of the *O* antigen, whereas the nonreacting bloods belong to the genotype *A_2A_2*. This is borne out by the fact that the cells of A_2B are nonreactive. It is therefore unfortunate that the name *anti-A_2* has been applied to this antibody.

The interrelationships of the subgroups may be summarized as shown in Table IV.

TABLE IV

Composition of the Subgroups

Group and Subgroup	Agglutinins Usually in Serum	Agglutinins Occasionally in Serum	Agglutination of Cells by Antibodies			
			Anti-A	Anti-A_1	Anti-O	Anti-B
A_1	anti-B	anti-O (in 0.6%)	always	always	never	never
A_2	anti-B	anti-A_1 (in 1-2%)	always	never	in 95%	never
A_1B	none	anti-O (in 3%)	always	always	never	always
A_2B	none	anti-A_1 (in 25-30%)	always	never	never	always
B	anti-A and anti-A_1	anti-O (very rare)	never	never	never	always
O	anti-A, anti-A_1, and anti-B	none	never	never	always	never

The Subgroup A_3. Once in several thousand bloods a specimen may be found that has the specificity A_3 or A_3B. This weakly-reacting agglutinogen was first described by Fischer and Hahn[6] and Friedenreich.[7] It is an inherited subgroup, like A_1 and A_2, and can be demonstrated by the procedure reported by Young and Witebsky.[8]

Subgroups of B. Many attempts have so far failed to demonstrate subgroups of the B agglutinogen.

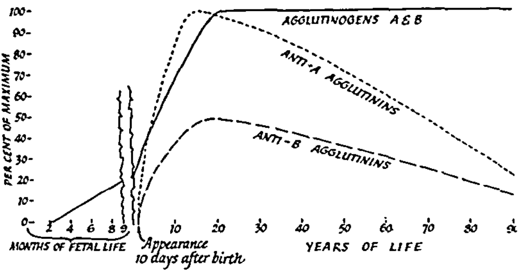
DEVELOPMENT OF BLOOD GROUPS DURING LIFE

Agglutinogens. The erythrocytes of the fetus contain the appropriate aggultinogens A or B of sufficient sensitivity to be recognized at about the thirty-seventh day of fetal life. The sensitivity increases steadily until, at the time of birth, it has attained about 20 per cent of the maximum found in later life. The sensitivity of the agglutinogens is tested by determining the greatest dilution of a given agglutinin serum which will cause the erythrocytes to be clumped. If the red cells from the baby at one month are agglutinated by the serum in a dilution of 1/20 and the cells at one year are clumped by the same serum diluted 1/80, the latter are said to be more sensitive than the former. The maximum sensitivity is attained at about 20 years and is sustained throughout the remainder of life.

Agglutinins. At birth the blood of the fetus contains no isohemagglutinins which are incompatible with the erythrocytes of the mother. Agglutinins are present in about one half of the newborn

infants but they disappear during the first ten days of postnatal life. It has been shown that these agglutinins are identical with those of the mother and presumably have diffused through the placenta. The relative insensitivity of the erythrocytes of the infant protect them from possible agglutination. The agglutinins of the infant begin to appear after ten days of life and increase in titer until puberty when the maximum is attained. Thereafter there is a slow decline in potency until old age.

DEVELOPMENT OF AGGLUTINOGEN SENSITIVITY & AGGLUTININ POTENCY



Clinical Significance. The low titer of agglutinins and the lack of sensitivity of the agglutinogens in the blood of the newborn have some clinical significance. The determination of the blood group is more difficult than in adults because the relative insensitivity of the erythrocytes requires highly potent grouping sera. This is particularly true with blood belonging to the subgroups A_2 and A_2B. The serum of an infant may not agglutinate the erythrocytes of a prospective donor of incompatible group. Actually, the infant may tolerate without reaction a single transfusion of incompatible blood. Thereafter isosensitization may increase the titer of the infant's agglutinins to such an extent that a transfusion with the same blood several weeks later may result seriously (p. 91).

In obstetric practice it is convenient to know that the newborn infant can tolerate blood from the mother unless the offspring has erythroblastosis fetalis. In the latter case, the mother's washed erythrocytes may be transfused with impunity (Chap. 8).

GROUP-SPECIFIC SUBSTANCES

Many investigators have demonstrated that when the agglutinogen A occurs in the erythrocytes a polysaccharide which inhibits

anti-A agglutinins is present in all body cells and fluids of the individual. A different carbohydrate inhibiting anti-B agglutinins is found in the tissues ,and fluids of persons whose erythrocytes contain the B agglutinogen. These inhibitors are termed *group-specific substances.* The agglutinogens A and B in the erythrocytes have not been shown to be carbohydrate in nature. Belkin and Wiener[9] have demonstrated that the agglutinogens are concentrated in the stromata of the red cells.

In the body cells the concentration of the group-specific substances is greatest in the stomach, pancreas, and submaxillary salivary glands. Lesser amounts are present in the myocardium, spleen, lungs, kidneys, prostate gland, liver, and parotid salivary glands. Small concentrations have been found in the small intestine and the testes. There is practically none in the brain.

In the body fluids the group-specific substances attain greatest concentration in the saliva, with diminishing amounts in the semen, amniotic fluid, erythrocytes, tears, and urine. There is none in the cerebrospinal fluid. Appreciable amounts of the A and B substances are present in the plasma of bloods of appropriate group.

Witbesky and Klendshoj[10] have isolated an O-specific substance from the gastric juice of persons belonging to group O.

Group-Specific Substances in Nature. Biologic materials which are closely related chemically and immunologically to the group-specific substances occur elsewhere in nature. Some lots of commercial pepsin and peptone contain the A substance. The erythrocytes of some rabbits possess an agglutinogen which is similar to the A antigen in man. The acetylated type-specific carbohydrate from Type I pneumococcus appears to be closely related to the A substance.[11] The A-specific substance can be isolated from the mucosa of the hog's stomach. The B substance is relatively rare in nature but it has been obtained, mixed with the A substance, in extracts of horse stomach.

Secretors and Nonsecretors

About 85 per cent of persons who possess the A or B substance in their tissues secrete it in the saliva, urine, tears, semen, gastric juice, and milk. The remainder do not possess this ability. Persons in the two categories are designated as *secretors* and *nonsecretors.* The ability to secrete the group-specific substance is inherited as a simple Mendelian dominant character, independent of the inheritance of blood groups or types. The chemical basis for the difference between the secretors and nonsecretors is due to the occurrence of the group-specific substance in two forms. A lipoidal fraction is present in the body cells of all persons of the blood group. An

additional water-soluble form occurs in the body fluids of secretors only and their secretions contain this substance.

NEUTRALIZATION OF AGGLUTININS WITH GROUP-SPECIFIC SUBSTANCES

In a heterospecific pregnancy (one in which the blood groups of mother and fetus are incompatible) the fetus is thought to be protected from the anti-A or anti-B agglutinins of the mother by the group-specific substances which are elaborated in the body of the fetus and diffuse into the amniotic fluid (p. 110).

When the plasma or sera of various blood groups are pooled, the titer of the natural agglutinins is reduced more than can be attributed to the dilution.[12] This is due to the fact that there is a certain amount of the group-specific substance in the plasma or sera of bloods whose cells contain the A and B agglutinogens.

Witebsky, Klendshoj, and Swanson[13] introduced the procedure of adding the A and B substances to group O blood to neutralize or suppress the agglutinins when it is to be transfused into persons of other groups. In their earlier work the A substance was isolated from commercial peptone and the B substance was derived from gastric juice of secretors belonging to group B. It was found that 25 mg. of the A-specific carbohydrate partially or completely neutralized the anti-A agglutinins in 500 ml. of citrated blood. The anti-B agglutinins were neutralized by an unstated amount of the B-specific substance. Later,[14] sources of the group-specific substance were found for large-scale production. The A substance was extracted from the gastric tissue of the hog and a mixture of the A and B substances was derived from the stomach mucosa of the horse. A mixture of the solutions of the A and AB substances can be prepared which is protein-free, without pyrogens, and safe for injection intravenously into man. There is no question that the titer of anti-A and anti-B agglutinins in the plasma of group O blood can be considerably reduced. Solutions of the A and B substances are conveniently employed in the laboratory to neutralize the anti-A and anti-B agglutinins in sera containing anti-Rh or anti-Hr agglutinins which are being tested or used as test sera. Witebsky's A and B substances can also be used as antigens in the production of blood grouping sera (p. 134).

INHERITANCE OF BLOOD GROUPS AND SUBGROUPS

The blood group of the individual is the result of chromosomal inheritance of the group-specific substances from one or both of the parents. Therefore it follows that the blood group of the individual does not alter during life because it is a characteristic of

all body cells. Instances of change in blood group have occasionally been reported but the observations are based on faulty technique in the identification of the blood groups or are caused by transfusions of large volumes of heterologous blood, which produce only ephemeral changes.

Studies of the inheritance of blood groups present unique opportunities to trace inheritance in human beings. Analyses of the blood groups in over 10,000 families have confirmed the hypothesis of Bernstein concerning the mechanism of inheritance of the agglutinogens according to Mendel's laws.

A-B-O Blood Groups

There are three allelic* genes *A*, *B*, and *O*. The genes *A* and *B* are equally dominant and gene *O* is recessive. In every somatic cell there is a pair of chromosomes in each of which is a single locus containing one gene for the blood group. The genes in the pair of chromosomes may be alike or different. If they are alike, the individual is *homozygous*, if different, he is *heterozygous*. All somatic cells of the individual contain identical patterns of chromosomes because they were originally derived by fission from a single fertilized cell. The gene pattern is termed the *genotype* whereas the resulting blood group, as demonstrated by test sera, is the *phenotype*. The four blood groups have the following composition:

Phenotype	Genotypes
AB	*AB*
A	*AA* and *AO*
B	*BB* and *BO*
O	*OO*

Groups A and B may be either heterozygous or homozygous. Group AB is always heterozygous whereas group O is always homozygous.

When two gametes are formed by the division of one body cell of the parent, the pair of chromosomes is separated so that each resulting germ cell receives only one gene which determines blood group. When the genotype is *AA*, all the germ cells contain gene *A*. If the genotype be *AO*, half the germ cells will contain gene *A* and half gene *O*. The union of germ cells of the two parents occurs by chance so that a parent of genotype *AO* may contribute either *A* or *O* to the offspring, but not both.

Laws of Heredity. Two laws of inheritance have been proved in accordance with Bernstein's theory: (1) The offspring cannot

* The term *allelic* here refers to the fact that all three genes carry characters pertaining to the A-B-O blood groups, although each gene may represent a different quality of the system.

possess the agglutinogen A or B, alone or in combination, except that it be inherited from one or both of the parents. (2) The parent of group AB cannot generate an offspring of group O, nor can a parent of group O give rise to a child of group AB. This is because the group AB is always heterozygous so that the *A* gene must come from one parent and the *B* gene from the other.

The only possible results from the matings of various blood groups are as follows:

Groups of Parents	Possible Groups of Children
O x O	O
O x A	O or A
O x B	O or B
O x AB	A or B
A x A	A or O
A x B	A, B, AB, or O
A x AB	A, B, or AB
B x B	B or O
B x AB	A, B, or AB
AB x AB	A, B, or AB

Inheritance of Agglutinins. There is no definite proof of the mechanism of inheritance of isohemagglutinins. Two theories have been advanced, either of which seems to fit the facts. Bernstein postulated the development in each individual of both anti-A and anti-B agglutinins but the incompatible antibodies are absorbed by the group-specific substances inherited with the blood group. The hypothesis of Schiff and Adelsberger assumes that the individual develops not only the group-specific substance which determines his blood group but also minute quantities of the others, not sufficient to absorb the characteristic agglutinin but strong enough to be antigenic. The characteristic agglutinin of the group is thus stimulated to develop.

THE SUBGROUPS

Sufficient data are available to confirm the hypothesis of Thomsen, Friedenreich, and Worsaae that the agglutinogens A_1 and A_2 are inherited by the distribution of four allelic genes A_1, A_2, B, and O. Genes A_1 and A_2 are dominant over gene O but gene A_1 is also dominant over A_2. In this arrangement the phenotypes and their component genotypes are as follows:

Phenotype	Genotypes
O	OO
A_1	A_1A_1, A_1O, and A_1A_2
A_2	A_2A_2 and A_2O
B	BB and BO
A_1B	A_1B
A_2B	A_2B

Laws of Heredity. According to the theories of Thomsen, Friedenreich, and Worsaae the following laws of inheritance have been proved to operate: (1) The agglutinogen A_1 cannot occur in the child unless derived from one or both of the parents. But since the phenotype A_1 can have the genotype A_1A_2, two parents of this genotype can produce an A_2 child. (2) The combinations of A_1B parent and A_2 child, and A_2 parent and A_1B child are impossible because the phenotype A_1B permits no genotype containing the gene A_2. (3) The matings A_1B x B and A_1B x A_1B cannot produce A_2B children because there is no genotype of A_1B which contains the gene A_2. (4) In the matings A_1 x O, A_1 x A_1, A_1 x B, A_1 x A_1B, and A_1 x A_2B the subgroups A_2 and A_2B are impossible in the child if it can be proved that a sibling from the parents is either B or O. The reason for this is that the genotypes of the B and O children must be either *BO* or *OO* so that a gene *O* must be derived from the A_1 parent, thus revealing the genotype of this parent to be A_1O which cannot give the offspring an A_2 gene.

The possible offspring from matings in the subgroups are as follows:

Subgroups of Parents	Possible Subgroups of Children
A_1 x O	A_1, A_2, or O
A_1 x A_1	A_1, A_2, or O
A_1 x A_2	A_1, A_2, or O
A_1 x B	A_1, A_2, B, A_1B, A_2B, or O
A_1 x A_2B	A_1, A_2, B, A_1B, or A_2B
A_1B x O	A_1 or B
A_1B x A_1	A_1, B, A_2B, or A_1B
A_1B x A_2	A_1, B, or A_2B
A_1B x A_2B	A_1, B, A_2B, or A_1B
A_1B x B	A_1, B, or A_1B
A_1B x A_1B	A_1, B, or A_1B
A_2 x O	A_2 or O
A_2 x A_2	A_2 or O
A_2 x B	A_2, B, A_2B, or O
A_2B x O	A_2 or B
A_2B x A_2	A_2, B, or A_2B
A_2B x B	A_2, B, or A_2B
A_2B x A_2B	A_2, B, or A_2B

Clinical Applications. The facts of inheritance of the blood groups have been employed in settling cases of disputed paternity and in the identifications of infants in nurseries. The determination of blood groups can never prove paternity but, in a certain number of cases, paternity can be disproved by the demonstration that the blood group of the putative father is impossible as the father of the child. Because the blood groups of identical twins must be the same, establishment of the fact that twins belong to different

groups proves them to be fraternal. Nothing is proved, however, if the groups are the same. Occasionally the demonstration of the blood group from a blood stain is of value in forensic medicine. It is not possible from serologic tests to prove that blood from two stains is identical.

RACIAL INCIDENCE OF BLOOD GROUPS

The frequency of the blood groups in various races of the earth has been studied extensively by anthropologists. The distribution of the subgroups has not yet received the same exhaustive attention. In general, the frequency of the A agglutinogen decreases as one goes from Western European stock eastward to the Pacific Ocean, whereas the B agglutinogen increases. The findings for many races have been tabulated by Wiener[11] to which the reader is referred for detailed information. It is convenient to remember the approximate incidence of the groups in Caucasians in the United States: group O, 45 per cent; group A, 40 per cent; group B, 10 per cent; and group AB, 5 per cent. This is contrasted with the proportions in the American Negro (Landsteiner and Levine): 44.2 per cent O; 30.3 per cent A; 21.8 per cent B; and 3.7 per cent AB. The incidence of the groups in the American Indian varies considerably among various tribes. In the Blackfeet and Bloods, according to Matson, Levine, and Schrader, it is: O, 22.8 per cent; A, 76.7 per cent; B, 0.0 per cent; and AB, 1.0 per cent. Allen and Korberr report the following distribution in the Navajos: O, 69.1 per cent, A, 30.6 per cent; B, 0.2 per cent; and AB, 0.0 per cent. The Chinese vary in different regions of their country but the results from several authors may be summarized as follows: O, 30–45 per cent; B, 20–35 per cent; A, 22–38 per cent; and AB, 6–11 per cent. There is also a similar range in the Japanese.

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    (b) Sobotka, H., Witebsky, E., Neter, E., and Schwarz, E. S : The relation of the blood group specific substance A to the type specific carbohydrate of pneumococcus type I. J. Infect. Dis. **60**:257, 1937.
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CHAPTER 5

The Blood Types M, N, and P

By Elmer L. DeGowin

After the discovery of the four blood groups in human beings it was soon realized that there were many other agglutinogens in the erythrocytes which were of little significance clinically because no natural agglutinins occurred to produce incompatibilities in blood transfusion. Nevertheless, systematic study of some of these agglutinogens has yielded information of some importance.

When systems of agglutinogens other than the A-B-O groups were recognized, it became desirable to make some distinction in terminology between them. In careful usage the elements of the A-B-O system now are referred to as *blood groups* and the procedure for identification is termed *blood grouping*. The systems M-N, P, Rh-Hr, and others are designated as *blood types* and the process of identification is called *blood typing*. There are also *subgroups* and *subtypes*.

THE M-N SYSTEM

Landsteiner and Levine[1] found that when erythrocytes from certain persons belonging to group O were injected into rabbits agglutinins developed which showed a certain specificity distinct from that exhibited by antibodies from the blood of others belonging to group O. With two antisera developed in rabbits by such a procedure it was demonstrated that all human beings can be classified in three types which depend on the reaction of their erythrocytes to the two sera. One serum is named *anti-M* and the other *anti-N*. Bloods in which the erythrocytes are agglutinated by anti-M serum are termed *type M* and those clumped by anti-N serum are *type N*. When the cells are agglutinated by both sera they are designated *type MN*. All human erythrocytes contain

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TABLE V

Racial Distribution of Types M, N, and MN (from Wiener[5])

Race	Authors	Number of Persons	Percentage		
			M	N	MN
Caucasians	Landsteiner and Levine	532	26 1	20 3	53 6
Caucasians	Wiener and Vaisburg	904	30 5	21 2	48 2
Negroes	Landsteiner and Levine	181	27 6	24 9	47 5
American Indians	Landsteiner and Levine	124	58 0	5 6	36.2
Japanese	Shigeno	202	30 3	23 9	45 8
Asiatic Indians	Wiener, Sonn and Belkin[6]	156	40 4	16 0	43 6
Mexican Indians	Wiener, Zepeda, Sonn, and Polivka[7]	98	61 2	3.1	35.7

pair of allelic genes *M* and *N*, both of which were dominant. Only three genotypes are possible, permitting three phenotypes:

Genotype	Phenotype
MM	M
NN	N
MN	MN

Theoretically, this mechanism should yield the parent-offspring combinations in Table VI.

TABLE VI

Possible Offspring from Parent Combinations in the M-N System

Parents	Percent Children		
	M	N	MN
M x M	100	0	0
N x N	0	100	0
M x N	0	0	100
MN x M	50	0	50
MN x N	0	50	50
MN x MN	25	25	50

From this hypothesis two laws of heredity should be true: (1) Neither agglutinogens M or N can appear in the child unless derived from one or both of the parents. (2) A parent of type M cannot have an offspring of type N and a parent of type N cannot have a child of type M. Wiener[5] states that no exceptions have been found to these rules in studies of over 15,000 children.

Clinical Significance of M-N System

Determination of the blood types in the M-N system is rarely of value in clinical medicine. Natural agglutinins against M or N

either one or the other or both of the agglutinogens *M* and *N*. The distribution of these factors occurs according to Mendelian laws and is independent of the inheritance of the A and B agglutinogens. The type of the individual in the M-N system remains the same throughout life. The agglutinogens have been demonstrated in the cells in the second month of intrauterine life and apparently are fully developed at birth.

Characteristics of Agglutinogens and Agglutinins

When potent antisera are employed, type N cells are nearly twice as sensitive to anti-N agglutinins as are cells belonging to type MN. A similar difference occurs when type M and MN cells are tested with anti-M serum although quantitatively the distinction is not so great.

The typing sera usually are rabbit *immune* sera. When they are produced by the injection of human erythrocytes, they contain species-specific antibodies in addition to the desired type-specific agglutinins. The former must be absorbed by contact with human erythrocytes which lack the agglutinogen specific for the type. For example, in case group O type N erythrocytes be injected into the rabbit, the resulting antiserum should be absorbed by group O type M cells to remove the species-specific agglutinins. If this procedure is performed properly, the resulting serum will be specific for type N cells.

Rarely an N agglutinogen is encountered which reacts very poorly with potent anti-N agglutinins. This has been termed *subtype* N_2. The N_2 agglutinogen is an extremely rare hereditary factor. It is undetected frequently by the usual anti-N agglutinin sera.

In general, no *natural* anti-M or anti-N agglutinins occur. So far, four exceptions to this statement have been found.[3] Three cases have been reported in which there was an *acquired* agglutinin which occurred as a result of isosensitization by blood transfusions. The antibody was anti-M in all seven cases.

Racial Distribution of Types M, N, and MN

The incidence of the various combinations in the M-N system varies in different races, although data are not so numerous as those for the A-B-O system. Some of the studies are summarized in Table V.

Inheritance of M and N Agglutinogens

Landsteiner and Levine[4] postulated that the distribution of types in the M-N system was explained by the inheritance of a single

(b) Wiener, A. S.: Blood groups and blood transfusion. Springfield, Ill., Charles C Thomas, 1945, 3rd Ed. (2nd printing), p. 219.
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have been extremely infrequent. The agglutinogens apparently are very poor antigens so that isosensitization by transfusion or pregnancy is not important. Identification of the types is valuable in cases of disputed parentage and in the study of the survival of erythrocytes after transfusion.

TYPES P+ AND P−

Landsteiner and Levine,[6] in their studies on the M-N system, encountered another distinct system of blood types in human beings. The erythrocytes of individuals can be characterized by the presence or absence of an agglutinogen which is designated as *P* and the blood is classified as *P+* or *P−*. This reaction is demonstrated best by the use of immune animal sera. Occasional human sera are encountered which contain weak anti-P agglutinins, either *natural* or *acquired*. No hemolytic transfusion reactions have been reported which could be attributed to their presence.[7] The P agglutinogen is probably inherited as a Mendelian dominant character. The occurrence of the P factor is not associated with the distribution of the agglutinogens A, B, M, N, Rh, or Hr. The racial distribution of the P factor has been studied to a slight extent. The data are summarized in Table VII.

TABLE VII

Racial Distribution of the P Agglutinogen

Race	Author	Number of Persons	Percentage	
			P+	P−
Caucasians	Wiener and Unger, quoted[8]	328	73 2	26 8
Negroes	Wiener and Unger, quoted[8]	73	97 7	2 3
Asiatic Indians	Wiener, Sonn, and Belkin[8]	20	70 0	30 0
Mexican Indians	Wiener, Zepeda, Sonn, and Polivka[9]	95	78 9	21 1

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and chemical structure of the serum or plasma which imparts to it the ability to cause the peculiar clumping. The abnormality usually lies in an increased concentration of fibrinogen or globulin of the plasma or in the addition to the plasma of a colloid such as gelatin or acacia. Since the reaction is not caused by a true antibody it has been designated by some as *pseudoagglutination*.

Specificity. The serum or plasma which has the property of causing rouleaux acts on red blood cells from any source, including the erythrocytes of the blood from which the serum or plasma is derived.

Effect of Dilution. The rouleau-forming property is readily destroyed by dilution of ½ or ⅓ with isotonic saline solution. This is in contradistinction to most true antibodies in the blood which are not so readily diluted out.

Effect of Temperature. Rouleau formation is maximal at 37° C. but also occurs in the cold. Storage of plasma or serum for a short time inactivates the rouleau-forming property.

Absorbability. The distinctive factor in the serum or plasma cannot be absorbed by the erythrocytes. This is in contrast to true antibodies.

Significance of Rouleau Formation

Interference with Crossmatching. Rouleau formation may be a confusing factor in crossmatching bloods before transfusion. The clumps may resemble true agglutination in appearance, macroscopically and microscopically, and must be differentiated from it by slight dilution of the plasma or serum. Furthermore, rouleaux may obscure the presence of true agglutinates which may be present coincidentally.

Sedimentation Rate. The formation of rouleaux is one mechanism by which red blood cells in suspension are caused to fall more rapidly than normal in a column. Measurement of this rate of fall, the *sedimentation rate*, was introduced into medicine by Fåhraeus.[1]

Clinical Occurrence. The concentration of fibrinogen in the blood plasma is increased in many infectious diseases. The plasma globulin may attain high values in many disorders including nephritis, advanced liver disease, and multiple myeloma. Serum or plasma from patients with these conditions is likely to cause rouleaux. The authors have encountered several instances in which the presence of intense rouleaux in crossmatching blood first directed attention to the diagnosis of multiple myeloma. Gum acacia or gelatin cause rouleaux when present in the blood in therapeutic concentrations (p. 189).

CHAPTER 6

Rouleaux, Cold Agglutination, and Similar Phenomena

By ELMER L. DEGOWIN

There are several phenomena which result in the clumping of red blood corpuscles which are not caused by the action of group- or type-specific agglutinins. The descriptions are confusing, partly because there has been no universal agreement on terminology. For the sake of clarity it is necessary to be somewhat arbitrary in definition.

ROULEAU FORMATION (PSEUDOAGGLUTINATION)

The serum or plasma of certain bloods has the property of causing human erythrocytes to assume the form of stacks of discs which look under the microscope like piles of coins. These rolls are known as *rouleaux* (French plural) and the process is termed *rouleau formation*. When the phenomenon occurs in moderate degree the microscopic appearance can scarcely be mistaken. If the reaction is more intense, however, large clumps of cells are formed which appear amorphous and resemble the agglutinates formed by the action of true isohemagglutinins. The typical piles of discs likewise do not occur when the erythrocytes have lost their biconcave form during storage. The presence of these atypical clumps sometimes seriously interferes with the correct interpretation of tests for cross-matching before transfusion (p. 189).

PROPERTIES OF SERA CAUSING ROULEAUX

The erythrocytes concerned in rouleau formation are normal in every respect. There is, however, an abnormality in the physical

Effect of Temperature. The maximum reaction is obtained from cold hemagglutinins at approximately 5° C. Most sera are inactive at 20° C. and rarely is one active at 37° C. In general, the broader the range of thermal activity, the greater the titer of the antibodies. When an erythrocyte suspension is mixed with a serum containing cold hemagglutinins, usually there is no clumping at room temperature. If the temperature is depressed to 5° C. the red corpuscles quickly agglutinate, frequently in large masses. Raising the temperature to 37° C. will cause the erythrocytes completely to separate from the clumps in a homogenous suspension again. The typical reactions may be repeated many times by raising and lowering the temperature.

Absorbability. The red blood cells of nearly all human beings will absorb cold hemagglutinins. Landsteiner[4] demonstrated that the agglutinins can be extracted in almost pure form by a simple procedure. The serum containing the antibodies is mixed with a suspension of red blood cells of any group and the mixture is placed in the refrigerator for one hour. The serum is then separated from the clumped cells by centrifuging in ice-packed cups and the supernant fluid is discarded. The addition of warm isotonic saline solution to the agglutinated cells causes the clumps to be dispersed and the agglutinins are released into the saline. The salt solution then contains the agglutinins in a high state of purity.

Cold Hemolysis. When a suspension of erythrocytes is clumped by cold hemagglutinins Stats[5] found that hemolysis can be produced by tapping the bottom of the test tube with the forefinger twenty to forty times. His experiments demonstrated that the destruction of erythrocytes by slight mechanical agitation only occurs when they are clumped by the action of the antibodies and the concentration of the erythrocytes exceeds 4 per cent. It is also necessary that the titer of the agglutinins be 1/3000 or greater. This type of hemolysis is not dependent on the presence of complement, in contradistinction to the Donath-Landsteiner phenomenon in which complement participates.

Clinical Significance of Cold Agglutination

A critical review of the subject of cold hemagglutination has been published by Stats and Wasserman[6] to which the reader is referred for many details. It seems probable that cold hemagglutinins *in low titer* can be found in most human beings and in many lower animals. The titer becomes greatly increased in certain pathologic states, in some of which it may assume a definite pathogenic significance. The antibody present in disease has not been demonstrated to be different qualitatively from that which

COLD HEMAGGLUTINATION (AUTOHEMAGGLUTINATION)

The phenomenon described under this heading has been known for many years and has been variously named *autohemagglutination*, *cold isohemagglutination*, *cold hemagglutination*, and *panagglutination*. Unfortunately some of these terms have been employed by certain authors to designate phenomena which are entirely unrelated to the subject under consideration. We prefer the term *cold hemagglutination* because it emphasizes the relation to temperature and is more descriptive than some of the others.

Most *natural* group-specific isohemagglutinins act more intensely at low temperature than at 37° C. but their reaction at the latter temperature is distinct. This is in contrast to cold hemagglutinins which react, with few exceptions, only at low temperatures and are completely inactive at body temperature. In blood containing cold hemagglutinins the erythrocytes are normal and the peculiar property resides in the plasma or serum. The absorbability and other properties of cold hemagglutinins classify them as true antibodies.

PROPERTIES OF SERUM OR PLASMA

It has been shown[2] that cold hemagglutinins are associated with the gamma globulin of the plasma. Because of their peculiar behavior it is possible to make nitrogen determinations on a solution which is nearly pure antibody. The antibody is a protein which has the electrophoretic mobility of gamma globulin. The antibody acts on a specific antigen in the erythrocytes. In a few cases cold hemagglutinins have been found in the blood of the pregnant woman but not in the child, which has led to the inference that the antibody does not cross the placental barrier.[6] The antibodies have been found occasionally in ascitic fluid, peritoneal exudate, cerebrospinal fluid, and blister fluid.

Specificity. Cold hemagglutinins cause clumping of the cells of the blood in which they occur. They also agglutinate the erythrocytes of nearly all human blood and of many other animals as well. The action on the blood of lower animals is somewhat variable;[3] rabbit cells seemed to have the greatest sensitivity of nine different species studied. Their wide field of action has caused some authors to apply the names of autoagglutinins or panagglutinins. In routine tests for cold hemagglutinins suspensions of human group O erythrocytes are employed to obviate the necessity for dealing with the anti-A or anti-B agglutinins.

Effect of Dilution. Cold hemagglutinins frequently act in great dilution so that titers of 1/1000 to 1/10,000 are not uncommon.

with high titers of cold hemagglutinins.[8] Stats and Bullowa[11] reported studies on a Negro who developed acrogangrene after exposure to cold. Thrombosis of the vessels occurred both in the fingers and toes. The serum of the patient contained cold hemagglutinins with a titer of 1/3200. The scleral vessels were observed with a slit lamp while the conjunctiva was irrigated with cold isotonic saline solution. Agglutination of the red blood cells was seen to cause segmentation of the columns of blood in the veins with consequent slowing of the blood stream. It was considered possible that when this process was prolonged it could cause thrombosis. When the patient's arm was immersed in cold water, hemoglobinemia occurred only in vessels in the chilled arm. It is to be emphasized that cold hemagglutinins have been found in only a few cases of Raynaud's syndrome but observations similar to this warrant search for the antibodies in all cases.

Venous Thrombosis. Several cases have been reported in which thrombosis of the veins was attributed to the presence of cold agglutinins in high titer.[6] The causal relationship has not been proved because there was no history of exposure to cold.

Acquired Hemolytic Anemia. It is an old observation that cold hemagglutinins are occasionally found in acquired hemolytic anemia. Stats and Wasserman[6] concluded that an etiologic relationship had not been proved. Instances have been reported in which the hemolytic anemia was cured by splenectomy but the cold hemagglutinins persisted.

Paroxysmal Cold Hemoglobinuria. Apparently there are at least two types of this disorder which should be sharply distinguished. One occurs exclusively in syphilitics and was extensively studied by Donath and Landsteiner.[12] Hemolysis occurs after exposure to cold but the blood does not contain cold hemagglutinins. A special isohemolysin occurs in the serum which is sensitized by cold. After sensitization, the hemolysin lyses erythrocytes at body temperature and only in the presence of complement. This phenomenon is termed *cold-warm hemolysis*.

Patients have been encountered in whom the Donath-Landsteiner test was negative and there were no signs of syphilis. Instead there were high titers of cold hemagglutinins. Exposure of the patient to cold resulted in hemolysis.

Cold Hemagglutinins and Blood Transfusion. Many transfusions of blood which contained cold hemagglutinins have been given without reactions attributable to the antibodies. In view of the evidence presented by Stats[5] it seems possible that hemolysis occasionally might be produced by excessive agitation of blood during storage when high titers of cold hemagglutinins were

is found in low concentration in health. In general, high titers of cold hemagglutinins occur in certain infections, in blood dyscrasias, and in peripheral vascular disorders.

Virus Pneumonia (Primary Atypical Pneumonia, Etiology Unknown). The presence of high titers of cold hemagglutinins in virus pneumonia was reported first by Peterson, Ham, and Finland[7] in 1943 and their findings have since been abundantly confirmed. Finland and his coworkers[8] studied the sera from 1069 persons with various diseases or no disease. Of 200 patients with virus pneumonia 68.5 per cent had cold hemagglutinins with a titer of 1/40 or higher. The antibodies were present in five of eleven cases of probable virus pneumonia and in four of seven cases of hemolytic anemia. Only 1.2 per cent of the remaining 851 persons with other disorders or controls had comparable titers, and in some of these virus pneumonia could not be excluded. The authors suggested that the development of cold hemagglutinins in this disease might be related to the causative agent and that testing for these antibodies could serve as a diagnostic aid.

The studies of Finland *et al.*[9] have shown that cold hemagglutinins are absent during the early part of the disease but they appear in the second or third week, usually when the patient is convalescing. The maximum titers are obtained between the middle of the second and the middle of the fourth weeks. Thereafter the titers drop rapidly and reach insignificant values between the fourth and sixth weeks. In most cases the maximum titer was between 1/40 and 1/1280, but in some a value of 1/10,000 was attained. In eleven of the 200 cases of virus pneumonia, hemolytic anemia occurred after the middle of the second week at the time when the maximal titer of cold agglutinins is to be expected. It was suggested that clod hemolysis from chilling of the peripheral blood may cause the anemia in these cases.

Tropical Diseases. Besides virus pneumonia, trypanosomiasis is one of the few infectious diseases in which the presence of cold agglutinins in high titer occurs with regularity. In 1911 Yorke[10] reported the appearance of cold hemagglutinins in animals with experimental trypanosomiasis. The agglutinins disappeared with recovery from the infection. Stats and Wassermann[6] do not regard as proved the significant occurrence of cold hemagglutinins in malaria. The antibodies have been noted frequently in animals with experimental relapsing fever but their presence in patients has not been observed.

Raynaud's Syndrome. In some patients with this disorder the vascular disturbance has been attributed, completely or in part, to the effect of cold on the peripheral blood vessels of persons

Contamination of blood serum or plasma by bacteria, such as *Corynebacterium Hektoenii*, may result in the production of a substance which agglutinates all red blood cells. This is prevented by the use of fresh or sterile plasma or serum, or the addition of 1/10,000 formalin, 1/1000 merthiolate, or 0.1 per cent acriflavine, brilliant green, or gentian violet.[14] These precautions should be taken in the use of grouping or typing sera.

FALSE AGGLUTINATION BY SERA FROM THE UMBILICAL CORD

Umbilical cord blood sera produce clumping of erythrocytes on glass slides which resembles true agglutination except that the clumps break up when the slides are allowed to stand. This has been shown to be due to contamination of the serum with Wharton's jelly.[15]

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present. Robertson and Rous[13] produced cold hemagglutinins in rabbits by repeated withdrawals of blood.

Hepatic Cirrhosis. Some writers have reported cold hemagglutinins occurring in cirrhosis of the liver. In a critical review of the evidence, Stats and Wasserman[6] concluded that sufficiently sharp differentiation was not made between cold hemagglutination and rouleau formation.

IRREGULAR COLD ISOHEMAGGLUTINATION

Agglutinins other than cold hemagglutinins occasionally are encountered in the study of plasma or serum at low temperatures. True cold hemagglutinins react equally well against the erythrocytes from all human beings and many animals (autoagglutinins). Most group-specific natural isohemagglutinins also react more intensely at low temperatures than at 37° C., but if they are present *in moderately strong titer, they will be detected at room or body* temperatures. The natural agglutinins anti-A_1 and anti-A_2 are usually weak and therefore should be differentiated from true cold hemagglutinins. It will be recalled (Chap. 4) that anti-A_1 agglutinins react against cells of the subgroups A_1 and A_1B whereas anti-A_2 antibodies clump the erythrocytes of subgroup A_2, and most group O bloods (from which fact they are also named *anti-O*). Anti-P agglutinins also react better at low temperatures and are usually weak in titer. Occasionally anti-Rh agglutinins are sufficiently strong so that they react not only at their optimum of 37° C. but also to a lesser degree in the cold. Still more confusing is the fact that cold agglutinins in strong titer may also react at 37° C.

BACTERIOGENIC AGGLUTINATION

The term *bacteriogenic agglutination* was suggested by Davidsohn and Toharsky[14] to designate clumping which is mediated by bacterial action on either erythrocytes or serum. Certain bacteria (genus *Corynebacterium* and some vibrios) may contaminate blood and form enzymes which transform a latent receptor, present in all erythrocytes, into an active agglutinogen which can unite with a specific agglutinin found in nearly all sera. Apparently this is a true antigen-antibody reaction because the agglutinin is absorbable by activated cells but not by uncontaminated erythrocytes. The phenomenon is called *panagglutination,* the *Hubner-Thomsen phenomenon,* or *T-agglutination.* The significance in blood grouping and crossmatching is obvious. Fresh or sterile preparations of blood cells and serum should be employed for these tests.

CHAPTER 7

The Rh-Hr Blood Types

By Elmer L. DeGowin

The experimental background for the discovery of the Rh-Hr system probably began in 1900 when Landsteiner[1] crossmatched the cells and serum of his laboratory assistants and demonstrated that human blood could be classified in several groups, depending on the presence or absence of one or both agglutinogens *A* and *B*. It was soon shown that there are four primary blood groups in the A-B-O system and that those lacking one or both of the antigens possess natural agglutinins, and sometimes hemolysins also, which are specific for the lacking agglutinogens. In the same year Ehrlich and Morgenroth[2] published their studies on the injection into goats of pooled laked goats' blood. They showed that the red cells of different goats contain individual antigens which can be detected by the antisera resulting from such injections. This was the first demonstration of the individuality of the red cells of a single species. Landsteiner believed that human red cells had a similar individuality and during the next forty years he and his pupils attacked the problem of elucidating these differences.

In 1928 Landsteiner and Levine[3] demonstrated that human erythrocytes can be classified in a second system which depends on the presence of one or both of the antigens *M* and *N*. A third independent system was soon described which depends on the presence or absence of the antigen *P*. The M, N, and P antigens are detected by the immunization of animals with red cells from human individuals and by testing the erythrocytes of human beings with the resulting antisera.

Landsteiner and Wiener[4] in 1940 reported studies in which rabbits were injected with the red cells of the Rhesus monkey. The re-

13. Robertson, O. H., and Rous, P.: Autohemagglutination experimentally induced by the repeated withdrawal of blood. J. Exper. Med. 27:563, 1918.
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in 1945. The next year Diamond found an antibody with the specificity of anti-Hr^0, whose existence had been predicted by Fisher from theoretical considerations.

NOMENCLATURE

As the facts accumulate which indicate the complexities of the antigen mosaic, the number of terms have necessarily multiplied. Disagreement as to the fundamental mechanisms of inheritance of the antigens has led to the proposal of several systems of nomenclature. Widest attention has been focused on two systems, the original one proposed by Wiener which he has subsequently expanded and revised to fit the newer discoveries, and another evolved by Fisher and used by Race and the English group of workers.

Wiener's Nomenclature

In Table VIII it will be noted that there are eight cell types and three antisera which are the reagents used in determining the types. The anti-Rh_0 serum agglutinates the cells of approximately 85 per cent of white persons and is frequently referred to as the "85 per cent serum." The anti-rh′ serum reacts with about 70 per cent and the anti-rh″ with 30 per cent of Caucasians. The cell types are named from the agglutinogens in the cells which are demonstrated by the three reagent antisera. All the names carry the letters *r* and *h*. The term *rh* is vocalized as "small r-h" or simply, "r-h." The types agglutinated by anti-Rh_0 bear the subscripts $_0$, $_1$, or $_2$, depending on whether they also react with anti-rh′ or anti-rh″. The terms Rh_1 and Rh_2 are merely shortened forms for Rh_0' and Rh_0''. The types not reacting with anti-Rh_0 have modifying superscripts.

TABLE VIII

Cell Types and Their Reactions

Cell Types	Antisera			Incidence in Caucasians %
	Anti-Rh_0 (85%)	Anti-rh′ (70%)	Anti-rh″ (30%)	
rh (Rh negative)	−	−	−	13 0
rh′	−	+	−	1 0
rh″	−	−	+	0 5
rh′ rh″	−	+	+	0.01
Rh_0	+	−	−	2 0
Rh_1 (Rh_0')	+	+	−	54 0
Rh_2 (Rh_0'')	+	−	+	15 0
Rh_1Rh_2 (Rh_0''')	+	+	+	14.5

sulting antiserum was found to agglutinate the erythrocytes of approximately 85 per cent of Caucasians, regardless of the presence or absence of the agglutinogens A, B, M, N, or P. They named this new antigen the *Rh factor*, from the word Rhesus, and classified bloods as being Rh positive or Rh negative from their reactions to this serum.

From its incidence in white persons Landsteiner and Wiener originally regarded the Rh factor as a single antigen which was inherited as a Mendelian dominant. The conception of the unity of the antigen soon had to be abandoned, however, because of the rapid advances in the knowledge of isosensitization. As patients were encountered who had been sensitized to the Rh antigens by transfusion or pregnancy, it was found that the acquired antibodies did not all give reactions coinciding with those of the classical anti-rhesus serum produced in rabbits. As a result the concept has been rapidly expanded until the Rh factor has now emerged as a veritable mosaic of antigens which are transmitted from parent to offspring by chromosomal combination. In papers published between 1940 and 1945 the nomenclature and the number of Rh types was in a state of flux so that the reader finds great difficulty in determining exactly the reagents and erythrocytes with which the authors were dealing. Since then, the issues have become more clear and the nomenclature, although still a subject of controversy, can now be understood.

REAGENT ANTISERA OF THE RH-HR SYSTEM

There are no naturally occurring antibodies in the Rh-Hr system so that all antigens in the system must be identified by acquired (immune) antibodies. Three antisera, all derived from human beings with isosensitivity, were early found to be essential in the characterization of the Rh types. They were named by Wiener: *anti-Rh⁰*, *anti-rh'*, and *anti-rh"*. In 1941 Levine, Javert, and Katzin[3] tested the antiserum in the blood of an Rh-positive woman which was found to agglutinate all Rh-negative cells and those Rh-positive cells which did not react with the anti-rh' serum. They named the antigen which was thus demonstrated the *Hr factor*, reversing the letters *r-h* to designate the reciprocal relationship to Rh agglutinogens. Race and Taylor[4] described a serum which, although stronger, was apparently similar to the anti-Hr reagent. The antigen which it defined was designated by the authors as *St* after the patient from whom the serum was derived. Wiener has adopted the name *anti-Hr'* for this antibody. Another agglutinin called anti-Hr", to use the nomenclature of Wiener, was described by Mourant

scripts. The symbols for genes are always italicized. In vocalizing, the terms are distinguished from the type names by the lack of *h's* in the gene characters.

The relations in Table IX plainly indicate the reciprocal nature of the reactions of each anti-Rh serum and its corresponding Hr antibody. But the reaction of a phenotype to one of the anti-Hr sera depends on *both components* of the genotype. As an example, consider two genotypes of the phenotype Rh_1: R^1R^1 and R^1r. Both give identical reactions to the three classic reagents anti-Rh_0, anti-rh′, and anti-rh″. The homozygous genotype R^1R^1 consists of two identical genes which do not react with anti-Hr′ serum and therefore are Hr negative. But the gene *r* of the genotype R^1r reacts with anti-Hr′, therefore the genotype R^1r is Hr′ positive by virtue of its "single dose" of the antigen. This is to be contrasted with the genotype R^0R^0 in which both genes react with anti-Hr′ and the type is said to have a "double dose" of the antigen. It has been reported that the difference between the single and the double dose of antigen can be detected serologically with weak anti-Hr′ serum by the degree of agglutination. Genotypes with the double dose are said to give a strong reaction whereas those with a single dose produce a medium or weak agglutination.

The nomenclature and the scheme of identification used by Wiener in Table X show the various components of the Rh-Hr system and their reactions to the six known antisera. It will be noted that reactions to the three anti-Rh sera determine eight Rh *types* which are further distinguished as *phenotypes*. Each phenotype may be determined by from one to five gene combinations, called *genotypes*. Certain genotypes of a phenotype may sometimes be differentiated by the reaction of the cells to the three anti-Hr sera. Since only anti-Hr′ serum is in diagnostic use, the subdivisions of the phenotype determined by this reagent have been given the names of *subtypes*. These are also phenotypes because they frequently embrace more than one genotype. Even with all three anti-Hr sera available, certain of the genotypes proposed by Wiener cannot be demonstrated serologically, but must be detected by study of the families in which they occur.

Fisher's Nomenclature

The nomenclature proposed by Fisher has been employed by Race[8] and many others. It should be pointed out that Fisher is interested primarily in problems of inheritance and has named only the antisera and the genotypes. He and other English workers still use the phenotype names suggested by Wiener,[9] or slight modifi-

TABLE IX

Theoretical Reactions with Genes

Genes	Antisera					
	Anti-Rh_0	Anti-Hr_0	Anti-rh′	Anti-Hr′	Anti-rh″	Anti-Hr″
r	−	+	−	+	−	+
r'	−	+	+	−	−	+
r''	−	+	−	+	+	−
R^0	+	−	−	+	−	+
R_1 ($R^{0'}$)	+	−	+	−	−	+
R_2 ($R^{0''}$)	+	−	−	+	+	−
R^y ($R^{0''}$)	−	+	+	−	+	−
R^z ($R^{0'\,''}$)	+	−	+	−	+	−

TABLE X

Rh Types, Subtypes, and Genotypes (Wiener)

Phenotypes (Rh Types)	Antisera			Genotypes	Antisera			Phenotypes (Rh subtypes)	Incidence in Caucasians %
	Anti Rh_0	Anti rh′	Anti rh″		Anti Hr_0	Anti Hr′	Anti Hr″		
rh	−	−	−	rr	+	+	+		13.0
rh′	−	+	−	$r'r'$	+	−	+	rh′rh′	0.01
				$r'r$	+	+	+	rh′rh	1.0
rh″	−	−	+	$r''r''$	+	+	−	rh″rh″	0.005
				$r''r$	+	+	+	rh″rh	0 5
rh′rh″	−	+	+	$r'r''$	+	+	+		0.1
Rh_0	+	−	−	R^0R^0	−	+	+		2.0
				R^0r	+	+	+		
Rh_1(Rh_0′)	+	+	−	R^1R^1	−	−	+	Rh_1Rh_1	20.0 (54.0)
				R^1r'	+	−	+		
				R^1r	+	+	+	Rh_1rh	34.0
				R^1R^0	−	+	+		
				$r'R^0$	+	+	+		
Rh_2(Rh_0″)	+	−	+	R^2R^2	−	+	−	Rh_2Rh_2	3.0 (15.0)
				R^2r''	+	+	−		
				R^2r	+	+	+	Rh_2rh	12.0
				R^2R^0	−	+	+		
				$r''R^0$	+	+	+		
Rh_1Rh_2(Rh_0′Rh_0″)	+	+	+	R^1R^2	−	+	+		14.5
				R^1r''	+	+	+		
				$r'R^2$	+	+	+		

Note. This omits the rare genes R^y ($r'\,''$) and R^z ($R^{0'\,''}$).

To present the fundamental reactions of the anti-Hr sera a theoretical table of *genes* must be considered. As symbols for genes Wiener uses *r* but drops the *h*. The modifying particles are super-

the original concept of Fisher. The combination of three letters was intended to denote the three genes in one of a pair of chromosomes whereas the modification actually represents the result of the reactions of the antigens of two chromosomes with two antisera which cannot possibly demonstrate the arrangement in a single chromosome. Furthermore the letter c represents a definite antigen to be demonstrated by agglutination with the serum anti-c and this test is not usually performed. Although the designation of a blood type as CDe gives the impression of being more exact than the Wiener phenotype Rh_1, actually it is not. When used in this sense, it could be the result of any of the genotypes CDe/CDe, CDe/Cde, CDe/cde, or/Cde/cDe.

The Wiener names are easier to vocalize and to recall to memory as units. On the other hand, the terms in Fisher's genotypes indicate exactly and explicitly the presence or absence of the various H genes (c, d, and e) whereas Wiener's system only implies them.

INHERITANCE

The hypothesis of Wiener as to the inheritance of the Rh-Hr system assumes a single locus for the Rh alleles in each of a pair of chromosomes. There is one gene in each locus. The genes in the two loci may be similar (homozygous) or dissimilar (heterozygous). They can only be derived from the germ cells of the parents. The eight possible genes which may reside in these loci are: r, R^0, r', r'', R^1, R^2, R^y, and R^z. There are thirty-six possible couplets of the eight genes, some of which have not yet been described. The resulting genotypes are designated by the symbols of the two genes of which they are composed, e.g., rr and $r'r$. Reference to Table X will show that these formulas are actually a form of shorthand because the presence or absence of the genes H^0, H', and H'' is implied. The complete expression of the genotype R^1r is therefore R^1H''/r $H^0H'H''$.

According to Fisher there are three pairs of allelic genes occupying three adjacent loci in each of a pair of chromosomes. At one pair of loci occur the combinations CC, Cc, or cc; at a second, DD, Dd, or dd; and at the third pair of loci EE, Ee, or ee. If no genes are linked in the chromosomal distribution, twenty-seven genotypes are possible. But additional antigens have already been discovered[12] at the first locus so that the following combinations are recognizable: CC, Cc, cc, CC^w, cC^w, and C^wC^w. Another factor seems to have been differentiated in the second position. Fisher[9] estimates that instead of eight types there may be eighteen or more, composed of 171 genotypes instead of thirty-six.

cations. Fisher originally designated the six diagnostic sera by Greek letters but the suggestion of Cappell has been widely adopted in which Latin letters are used to correspond with the genes against which they react. The equivalents are:

Names of Antisera

Wiener's nomenclature:	Anti-rh′	Anti-Rh_0	Anti-rh″	Anti-Hr′	Anti-Hr_0	Anti-Hr″
Fisher's nomenclature:	Anti-C	Anti-D	Anti-E	Anti-c	Anti-d	Anti-e

The equivalent names of the genes in the two nomenclatures are:

Names of Genes

Wiener's nomenclature:	r′	R^0	r″	H′	H^0	H″
Fisher's nomenclature:	C	D	E	c	d	e

Fisher conceives of each pair of chromosomes as bearing a triplet of genes which determine the Rh-Hr system. The corresponding gene in each of the pair of chromosomes forms one of the following combinations: CC, Cc, or cc; DD, Dd, dd; EE, Ee, or ee. The equivalent genotype nomenclature is:

Names of Genotypes

Wiener	*Fisher*	*Wiener*	*Fisher*	*Wiener*	*Fisher*
rr	cde/cde	R^0r	cDe/cde	R^2r''	cDE/cdE
r′r′	Cde/Cde	R^1R^1	CDe/CDe	R^2r	cDE/cde
r′r	Cde/cde	R^1r'	CDe/Cde	R^2R^0	cDE/cDe
r″r″	cdE/cdE	R^1r	CDe/cde	$r''R^0$	cdE/cDe
r″r	cdE/cde	R^1R^0	CDe/cDe	R^1R^2	CDe/cDE
r′r″	Cde/cdE	$r'R^0$	Cde/cDe	$r'R^2$	Cde/cDE
R^0R^0	cDe/cDe	R^2R^2	cDE/cDE	R^1r''	CDe/cdE

The longest genotype name in the Wiener nomenclature is vocalized as "R-two-prime, R-two-prime" whereas its equivalent in the Fisher system is pronounced "Small-c-small-d-large-e, small-c-small-d-large-e." For a summary of the other proposed nomenclatures for the Rh system the reader is referred to Potter.[10]

It seems probable that either nomenclature can be adapted to the future discoveries in the inheritance of the Rh-Hr system. The Wiener nomenclature has phenotype names which can be derived from the results of objective serologic tests with the available reagents, without any implication as to the exact genotype. This is a definite advantage in routine diagnostic work.

Some American workers have attempted to remedy the lack of phenotype names in the Fisher system by adopting a convention which has only tacitly been admitted. When a phenotype name is desired for the type Rh_1, for example, it is called CDe from its reaction to anti-C and anti-D sera and the lack of agglutination with anti-E serum. This involves two subtle changes in meaning from

long periods. When Rh antisera became available for testing, data accumulated as to the incidence of the various subtypes in different races. Most of the older work was performed with a single antiserum, either anti-Rh_0 or anti-Rh_0', so that all the types were not differentiated and the term *Rh negative* varied slightly in meaning. The reported data have been assembled by Potter.[10]

The highest incidence of rh occurs in Caucasians. The occurrence is between 12 and 17.7 per cent in a group of peoples consisting of American Caucasians, English Caucasians, Canadians, Australians, Brazilians, Argentinians, and Uruguayans. An incidence of 24.6 per cent has been reported in Belgians and 33.6 per cent in Basques. In an intermediate group of peoples in which the incidence of rh is from 4 to 9.8 per cent are found the Canadian Jews, Chileans, Salvadorians, American Negroes, Asiatic Indians, and South American Bantus. A third group, in which rh occurs in from 0 to 1.1 per cent, is composed of Brazilians (another series), American Indians, Hindus, Chinese, Japanese, Eskimos, Indonesians, Papuans, Australian aborigines, Maoris, Fijians, and Burmese.

The data available on the racial distribution of all eight types of Rh are less comprehensive. Some of them are summarized in Table XI.

TABLE XI

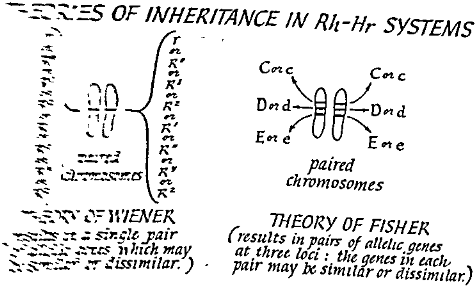
Racial Incidence of Rh Subtypes

Population	Number of Persons	Percentage of Rh Types							
		rh	Rh_1	Rh_2	Rh_1Rh_2	Rh_0	rh′	rh″	rh′rh″
Caucasian (American)[13]	1468	13.3	54.4	13.7	15.0	2.4	1.0	0.2	0
Caucasian (English)[14]	927	14.7	54.8	12.2	13.6	2.5	0.6	1.3	0
Caucasian (Australian)[15]	350	14.8	54.0	12.5	16.5	0.5	0.8	0.5	0
Negroes (American)[16]	223	8.1	20.2	22.4	5.4	41.2	2.7	0	0
Asiatic Indians[18]	156	7.1	70.3	5.1	12.8	1.9	2.6	0	0
Chinese[19]	132	1.5	60.6	3.0	34.1	0.8	0	0	0
Japanese[20]	150	1.3	37.4	13.3	47.3	0	0	0	0.7
Mexican Indians[21]	98	0	48.0	9.2	41.8	1.0	0	0	0
Filipinos[17]	100	0	87.0	2.0	11.0	0	0	0	0

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The differences between the two theories of inheritance will probably be resolved when further studies of the blood types in families are made, particularly in non-European peoples in whom [illegible] is rare. Data from these studies will undoubtedly indicate whether R^1 and R^2 are linked regularly ($R^0 + r'$ and $R^0 + r''$) as single genes in accordance with the Wiener concept, or whether they occasionally break up into their components (R^0, r', and r'') as the hypothesis of Fisher would seem to imply.



Wiener's assumption of two allelic genes is the simpler mathematically. The available data on inheritance apply equally well in the explanation of either hypothesis, but the burden of proof would seem to rest with the proponents of Fisher's concept. The theory of three adjacent loci on the same chromosome can only be proved when instances of accidental crossing over of genes are demonstrated. None have thus far been found.

Suffice it to say that this is a question for the geneticist, and the application of the findings to clinical medicine seems at present a little obscure. It is probably unnecessary that the nomenclature [illegible]

RACIAL DISTRIBUTION OF RH TYPES

Previously it had been demonstrated that the incidence of the antigens A, B, M, and N varies greatly in different races of men and that the occurrence of any one antigen is approximately the same in a race for long periods of time, as judged from the study of racial groups which have been isolated from the main body for

CHAPTER 8

Isosensitization

By Elmer L. DeGowin

The study of the Rh antigens particularly has led to a broader concept of human immunity. It is now established that a human being can be sensitized to an antigen in the erythrocytes of another. After sensitization to an isohemagglutinogen has occurred subsequent contact with the antigen causes reactions with the antibodies of the sensitized individual. The condition induced in man by the production of antibodies from contact with antigens in other human erythrocytes has been termed *isoimmunity* and the process of the development of the state designated as *isoimmunization.* The prefix *iso-* implies derivation from the same species. The word *immunity* is employed in its extended sense to denote a condition in which antibodies of any kind develop. In the minds of many, however, the word immunity carries the original implication of protection, whereas the state under consideration is potentially dangerous for the individual because of the nature of the antibody reaction. Therefore it has been proposed[1] that the terms *isosensitization* and *isosensitivity* be employed. These can leave no doubt as to the clinical vulnerability which the state entails for the individual.

Two Routes of Isosensitization. The agglutinogens in the red cells of one person may come in contact with the blood of another and produce isosensitization by either, or both, of two routes. The erythrocytes may be introduced by blood transfusion. Or, during pregnancy, the red cells of the fetus may cross the placental barrier and enter the circulation of the mother. The reverse course, from the mother to offspring, has not been demonstrated to be of clinical significance.

Two Manifestations of Isosensitivity. Once the individual has become sensitized, by whichever route, the agglutinins and other

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immunization to the stronger occurs to the exclusion of the weaker. Wiener[4] analyzed data which were interpreted to show that such a phenomenon probably occurs in isosensitization during pregnancy. When Rh sensitization was present, the incidence of compatibility in the A-B-O system between the blood of the mother and fetus was higher than could be accounted for by chance. It was assumed that the absence of the more potent antigens A or B permitted the weaker Rh antigens to sensitize. When the erythroblastotic fetus belonged to Rh_1, the rh mother sometimes formed antibodies against only Rh_0 although anti-rh′ agglutinins would also have been expected. This is explaind by the assumption that the stronger Rh_0 antigen suppresses the antigenicity of rh′.

ANTIBODIES IN ISOSENSITIZATION

When isosensitization occurs the body responds either by the enhancement of *natural antibodies* or by the production of *acquired (immune) antibodies.* For example, if a group A recipient, possessing natural anti-B agglutinins, receives an injection of group B cells, the titer of the antibodies rises to a high value. But when an rh individual receives Rh_0 antigen, he may respond by the development of acquired Rh_0 antibodies.

Enhancement of Natural Antibodies

During isosensitization the titer of natural antibodies may be greatly increased. The anti-A and anti-B agglutinins have been studied most extensively because they are far the most common. It has been assumed that the natural agglutinins are not changed qualitatively when they are enhanced during isosensitization. The recent studies of Boorman and Dodd[5] make it seem likely that this cannot be accepted without modification. They demonstrated that natural agglutinins which had not been enhanced by isosensitization showed no difference in potency against test cells suspended in either saline or serum. When the anti-A or anti-B agglutinins had been enhanced by the injection of erythrocytes containing the appropriate A and B agglutinogens, the titer of the agglutinins against saline-suspended test cells was increased, but a still further augmentation could be demonstrated when the test cells were suspended in serum. This seems to indicate the development of an antibody of a different order than the natural agglutinins.

Transfusion of Blood of Heterologous Groups. A few recipients who have been transfused with blood of incompatible group have been studied. If a person of Group O receives blood of group A and

antibodies thus formed react when the subject again contacts the specific antigen. The nature of the reaction depends upon the route by which the shocking dose of antigen is introduced. If blood containing the antigen is injected in a transfusion, intravascular hemolysis of the donor's cells occurs. When the antigen-bearing erythrocytes enter the blood of the mother from the fetus, she is not affected, but her antibodies produce hemolysis of the cells of the fetus.

ANTIGENS CAUSING ISOSENSITIZATION

All isohemagglutinogens which have so far been studied in human blood must be considered as potential antigens in transfusion and pregnancy. The data which have been accumulated indicate that some agglutinogens are much more antigenic than others. There are also differences in antigenicity which depend upon whether the antigen is introduced by transfusion or through the placenta. Although exact data are not available the comparison in Table XII will give some perspective.

TABLE XII

Relative Antigenicity of Agglutinogens

Antigen	Route of Sensitization	
	By Transfusion	In Pregnancy
A to A-negative persons	Almost 100%	Rare
B to B-negative persons	Almost 100%	Rare
M to M-negative persons	Rare	Unreported
N to N-negative persons	Unreported	Unreported
P to P-negative persons	Very rare	Unreported
Rh to Rh-negative persons	5 to 30%	About 5%
Hr to Hr-negative persons	Rare	About 0.3%

Clinically isosensitization varies greatly among the Rh types. Rh_0 is by far the most frequently antigenic of the Rh factors. For this reason persons belonging to rh′, rh″, or rh′rh″ are nearly as susceptible to sensitization to Rh_0 as are those who belong to rh.[2] A person who belongs to type Rh_1 rarely becomes sensitized to rh″ cells, and one who is Rh_2 is seldom immunized to rh′.[3] For this reason Wiener suggested that anti-Rh_0 serum be employed to type prospective recipients for transfusion. This classifies rh′ and rh″ cells as Rh negative so that persons of these types will receive rh blood.

Competition of Antigens in Isosensitization. When an animal is injected with a strong and a weak antigen simultaneously,

immunization to the stronger occurs to the exclusion of the weaker. Wiener[4] analyzed data which were interpreted to show that such a phenomenon probably occurs in isosensitization during pregnancy. When Rh sensitization was present, the incidence of compatibility in the A-B-O system between the blood of the mother and fetus was higher than could be accounted for by chance. It was assumed that the absence of the more potent antigens A or B permitted the weaker Rh antigens to sensitize. When the erythroblastotic fetus belonged to Rh_1, the rh mother sometimes formed antibodies against only Rh_0 although anti-rh′ agglutinins would also have been expected. This is explaind by the assumption that the stronger Rh_0 antigen suppresses the antigenicity of rh′.

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Transfusion of Blood of Heterologous Groups. A few recipients who have been transfused with blood of incompatible group have been studied. If a person of Group O receives blood of group A and

survives the resulting hemolysis, the titer of the anti-A agglutinin of the recipient is first depressed for a few hours because antibodies are absorbed by the transfused erythrocytes. The potency then gradually increases during the next ten or twelve days so that the titer attains values many times the original.

In Wiener's case[6] the group O recipient inadvertently received a transfusion of 500 ml. of group B blood. The patient experienced a chill, fever, hemoglobinuria, and hemoglobinemia, but recovered. The titer of the anti-B agglutinin rose from 1/1, two hours after transfusion, to 1/512 on the thirteenth day. The titer of the anti-A agglutinin remained at 1/32 throughout. Mollison and Young[7] studied the blood of a young woman of group O who received 360 ml. of group B blood, 1500 ml. of pooled plasma, and 100 ml. of four times concentrated pooled serum. The titer of the anti-A agglutinin rose from 1/64 to 1/65,536 on the tenth day. The potency of the anti-B agglutinin was augmented from a titer of 1/2 to 1/262,144 on the ninth day. Both agglutinins had resumed normal concentrations by the fifty-second day after transfusion. The small amounts of A and B substance in the pooled plasma and serum had apparently acted as antigens, in addition to the B agglutinogen in the donor's blood. These authors made observations on two group O subjects, each of whom was injected with 100 ml. of four times concentrated serum containing the group-specific substance A. The titer of the anti-A agglutinin underwent temporary reduction after injection, then the potency increased to a titer of 1/2048 between the sixth and tenth day. There was a slow fall to normal levels in thirty-six days. The titer of the anti-B agglutinin was relatively constant and normal throughout the period of observation.

The enhancement of natural agglutinins during transfusion has dangerous implications, as illustrated by the case observed by one of us. The blood of an infant, several weeks of age, was erroneously concluded to belong to group AB and appeared to be compatible with that of a group AB donor when crossmatched. The donor gave blood to the child without accompanying reaction. Two weeks later, in preparation for another transfusion from the same donor, it was found that the recipient's serum agglutinated group AB cells from several sources, Stronger grouping sera demonstrated that the recipient's cells belonged to group B. Apparently the anti-A agglutinin had been enhanced by the injection of the A agglutinogen in the first transfusion It would not have been safe to conclude that the blood of the same donor could be employed in a second transfusion because it had not produced a reaction initially. Thalhimer[8] has reported a similar case.

Transfusion of Blood of Heterologous Subgroups. A few cases have been reported in which the natural irregular agglutinins anti-A_1 and anti-A_2 (anti-O) apparently have been enhanced by transfusions of blood containing the appropriate A_1, A_2, or O antigen.[15,16] In most cases, however, it seems uncertain as to whether the irregular agglutinin was previously present and augmented as a result of isosensitization, or whether it was an acquired antibody.

Injection of Group-Specific Substances. Deliberate isosensitization of human beings has been employed for the enhancement of natural antibodies. Witebsky, Klendshoj, and McNeil[9] devised a method for increasing the titer of anti-A and anti-B agglutinins in the preparation of blood grouping sera (p. 134). Donors of suitable blood group are injected intravenously with small amounts of extracts of hog and horse stomach which contain the A and B group-specific substances. In approximately three weeks the titer of the natural agglutinins is usually greatly increased and the blood serum is then employed for grouping tests. Wiener, Soble, and Polivka[10] suggested the injection intramuscularly of extracts of saliva from secretors of appropriate blood group to enhance the titer of agglutinins for grouping sera. (p. 134).

Nonspecific Stimulation of Natural Agglutinins. Davidsohn[11] observed an increase in the titer of natural agglutinins after the development of serum sickness from horse serum.

Stimulation of Natural Agglutinins During Pregnancy. When the blood of the fetus contains either the A or B agglutinogen which is not present in the mother's blood, the titer of the anti-A or anti-B agglutinin of the mother's plasma becomes augmented during pregnancy.[12] This phenomenon apparently occurs with great regularity and is not to be confused with the instances in which an excessive rise in titer is associated with the development of erythroblastosis fetalis induced by the natural antibody of the mother.

Production of Acquired Antibodies

In isosensitization acquired antibodies develop when no specific natural antibodies exist for the antigen. In general they are specific for the antigens which stimulate their production. Several orders of acquired antibodies have so far been identified and it seems probable that many more have not yet been differentiated.

Acquired (Immune) Agglutinins (Synonyms: Complete Antibodies, Early Immune Antibodies, Bivalent Antibodies, First Order Antibodies). Like natural isohemagglutinins, these are characterized by their ability to clump specific antigenic erythrocytes.

Agglutination of Cells Suspended in Saline Solution. Acquired agglutinins cause clumping of specific erythrocytes when the latter are suspended in a solution of sodium chloride. This property is similar to that of natural agglutinins but differentiates them from blocking antibodies (glutinins) which produce little or no agglutination of saline-suspended red cells.

Optimum Temperature of Action. Most acquired agglutinins produce optimal clumping of red cells at approximately 37° C. (warm agglutination). This is in contrast to the natural agglutinins anti-A and anti-B which react well at room temperature but best at about 5° C. (cold agglutination).

Cohesiveness of Agglutinates. Acquired agglutinins produce clumps of erythrocytes which are small and readily shaken apart, in contrast to anti-A and anti-B agglutinins which form agglutinates which are inseparable with ordinary handling. This property makes more difficult the technical manipulation and interpretation of tests for acquired agglutinins.

Absorbability of Acquired Agglutinins. Although natural agglutinins are readily absorbed by suitable contact with erythrocytes containing the specific agglutinogens, acquired agglutinins are absorbed with difficulty and but little by the usual methods.

Specificity of Acquired Agglutinins. Acquired agglutinins are specific for the antigen which stimulates their production. In human isosensitivity agglutinins for Rh and Hr are most frequently encountered. Perhaps the most common is anti-Rh_0' (anti-C anti-D), which is a combination of anti-Rh_0 and anti-rh'. The next in frequency is anti-Rh_0 (anti-D). Occasionally sera of the specificity anti-Rh_0'' (anti-D anti-E), anti-rh' (anti-C), and anti-rh'' (anti-E) are found. Of the Hr sera, anti-Hr' (anti-c) is the most common, while a single instance of the occurrence of each of the sera anti-Hr_0 (anti-d) and anti-Hr'' (anti-e) has been reported.

As previously noted (p. 93) it seems probable that in some of the instances in which an irregular agglutinin has been found after isosensitization the antibody is probably an acquired type.

Acquired Agglutinins in Early Isosensitivity. It is now established that acquired agglutinins are the earliest antibodies to appear during the course of isosensitization, and their presence without accompanying glutinins (blocking antibodies) usually indicates a relatively mild degree of immunity.

Glutinins (Synonyms: Blocking Antibodies, Inhibitor Antibodies, Incomplete Antibodies, Hyperimmune Antibodies, Univalent Antibodies, Second Order Antibodies). In the earlier studies of isosensitization during pregnancy the expected anti-Rh agglutinins

could not be demonstrated in about half of the Rh-negative mothers of Rh-positive erythroblastotic offspring. Wiener[17] and Race[18] independently discovered the presence of another acquired antibody in the blood of Rh-sensitized mothers which united with Rh-positive cells but did not cause clumping with the usual tests. The antibody has been given various names to emphasize some of its characteristics.

The Blocking Phenomenon. When serum containing glutinins (blocking antibodies) specific for the Rh_0 antigen, for example, is incubated with Rh_0 cells suspended in saline solution, no clumping is observed. If potent anti-Rh_0 agglutinins be added to the serum-cell mixture and again incubated, the cells are not agglutinated by the specific antiserum. Wiener conceives the mechanism of this reaction to be a coating of erythrocytes by the glutinins so that subsequent addition of agglutinins does not effect the characteristic clumping of red cells.

Influence of Saline and Protein Solutions on Clumping. Diamond and his coworkers[19,20,21] demonstrated that although blocking antibodies do not produce clumping of the specific saline-suspended erythtocytes, agglutination is accomplished when the test cells are suspended in plasma, serum, or serum albumin (either human or bovine). This discovery led to the perfection of delicate tests for the demonstration of glutinins, such as the slide test of Diamond and Abelson (p 163) and the conglutination test of Wiener (p. 176).

The assumption was made by Wiener[22] that there is a special substance in plasma proteins which is similar, if not identical, to the X-protein described by Pedersen. Specific erythrocytes coated by the glutinins absorb the X-protein (conglutinin) and become clumped. Wiener does not consider that this phenomenon is true agglutination and therefore terms it *conglutination.* Unlike agglutination, the readings cannot be improved by centrifugation and the sediment pattern of the erythrocytes cannot be relied upon as an indication of a reaction, so that microscopic examination is imperative. Wiener *et al.*[23] reported studies in which the maximal potency of glutinins was attained by using four parts of pooled plasma with one part of human serum albumin (25 per cent). Mixtures of two parts of human serum albumin (12.5 per cent solution) and one part of human gamma globulin (4.6 per cent) gave optimal results when neither component alone was very active. This is considered evidence in favor of the action of the X-protein which is formed according to the following equation:

$$\text{Albumin} + \text{gamma globulin} \rightleftarrows \text{X-protein}$$

Boorman and Dodd[5] studied the enhancing effects of sixty-seven different human sera on agglutination produced by one serum containing Rh antibodies and found that the various sera possessed great variability in this enhancing property. They concluded that this effect is due to the presence of a specific factor which is found in varying amounts in all normal sera. Apparently this is confirmed by the findings of Witebsky *et al.*[56]

Stability and Absorbability of Glutinins. The ability of protein solutions to potentiate the clumping of red cells by glutinins is not impaired by heating the solutions at 56° C. for thirty minutes.[5, 24] The glutinins seem to be more readily absorbed, particularly at 0° C., than are acquired agglutinins.[25]

Optimal Temperature of Action of Glutinins. Like the acquired agglutinins, glutinins react maximally at 37° C.

Specificity of Glutinins. Most glutinins have the specificity anti-Rh_0, although occasionally rh′ or rh″ antibodies are encountered. Frequently during the course of isosensitization to two Rh antigens the serum contains an Rh_0 glutinin and an rh′ or rh″ agglutinin. The serum may then be employed as an anti-rh′ or anti-rh″ typing serum, provided the erythrocytes to be tested are suspended in saline solution to preclude the conglutination caused by the Rh_0 glutinins.

Boorman and Dodd[5] found that the action of natural agglutinins anti-A and anti-B was not enhanced by the addition of normal human serum which contained no antibodies specific for the test cells. If the titer of the agglutinins was increased by the injection of the subject with A and B substance, the potency of the agglutinins was still further enhanced by the addition of normal human serum. This would seem to indicate the presence of glutinins with a specificity of anti-A and anti-B.

In his original paper on blocking antibodies Wiener[17] predicted that their presence might be discovered in immunization to antigens other than isoagglutinogens. This has recently been achieved by Griffitts[26] who demonstrated the presence of strong anti-Brucella blocking antibodies in the sera of patients with brucellosis in whom agglutinins for the bacteria could not be detected by the usual methods.

Acquired Isohemolysins. Natural agglutinins frequently are associated with isohemolysins of the same specificity. The existence of acquired isohemolysins is the subject of some doubt. Levine and Polayes[27] reported the presence of a hemolysin associated with an agglutinin of atypical specificity in the blood of a woman who had a transfusion reaction twelve days post partum. The agglutinin and hemolysin were not specific for the antigens A, B, M, N, or Rh. The authors assumed that the antibody had been acquired by

isosensitization during pregnancy. Hill and Haberman[28] reported the demonstration of anti-Rh hemolysins by incubation of the serum containing anti-Rh agglutinins with type-specific erythrocytes for twenty hours. The amount of free hemoglobin released was compared with that in control specimens. The evidence is not conclusive because the small differences in the amount of hemolysis could be due to artifact and it seems unlikely that true hemolysins should be so slow in action.

Other Acquired Antibodies. Undoubtedly there are still other orders of acquired antibodies which can be characterized in isosensitization by indirect methods. Coombs and his coworkers[29] demonstrated that erythrocytes which had adsorbed acquired antibodies (such as anti-Rh) could be washed thoroughly and then clumped by the addition of anti-human globulin serum. Although this phenomenon was not antigen-specific, it did differentiate between cells sensitized by natural antibodies and those which had been subjected to acquired antibodies. Hill and Haberman[28] proposed that the antibodies demonstrated by the anti-human globulin serum be considered as third order antibodies. Witebsky[30] recently presented evidence of other orders of antibodies in isosensitivity which have some of the characteristics of blocking antibodies.

Persistence of Acquired Antibodies

While the evidence is incomplete, it seems probable that antibodies acquired during isosensitization in transfusion or pregnancy persist for many years, if not permanently. They may disappear from the blood stream only to reappear when the individual has further contact with the specific antigen.

Anamnestic Reactions

Literally, the adjective *anamnestic* means "recalling to memory." It has been applied in immunology to the phenomenon in which previously developed antibodies, which have disappeared from the blood stream, later reappear because of the action of a nonspecific stimulus. Occasionally this is noted in the mother, previously sensitized to an Rh antigen, who later bears an offspring whose blood lacks the specific antigen to which she is sensitized. Nevertheless, the antibody which she formerly acquired reappears in her blood during the pregnancy, although the fetus is normal.

ISOSENSITIZATION AND TRANSFUSION

Blood transfusion bears two relations to isosensitization: (*a*) the sensitizing red cells may be introduced into the body of the susceptible person by transfusion, and (*b*) the shocking dose of antigen

may be injected into the body of the sensitized individual by transfusion and produce intravascular hemolysis.

ISOSENSITIZATION DUE TO BLOOD TRANSFUSION

Incidence of Isosensitization from Blood Transfusion. Many factors influence the occurrence of isosensitization in a single series of transfusions. The relative antigenicity of the various agglutinogens, from the greatest to the least, may be stated in the following order: A and B, Rh_0, Hr′, rh′ and rh″, O and A_2, A_1, M, P, and N. The incidence of isosensitization depends upon such factors as: (1) If transfusions were given regardless of the blood group of the donor and recipient in the A-B-O system, the number of cases of isosensitization would be prohibitive. This type of immune response was minimized many years ago when the cause of intergroup reactions was discovered and the practice of using homologous groups of blood for transfusion was introduced. (2) The development of anti-O (anti-A_2) agglutinins would be higher in a series in which many transfusions of group O blood were given as universal donor blood. (3) The proportion of recipients who have been pregnant in the series will affect the incidence of reactions from isosensitization. (4) If Rh-positive blood is given to Rh-negative recipients routinely, the incidence of isosensitivity will be greater than if homologous Rh types are used. (5) When methods of preliminary crossmatching are suitable to detect Rh antibodies, the number of reactions due to isosensitivity is low. (6) The number of transfusions given to each recipient has a direct bearing on the number of persons sensitized. (7) The elapsed time between transfusions has a bearing on the reactions from isosensitivity.

In a study of over 3000 blood transfusions Wiener *et al.*[31] encountered two intergroup reactions due to the enhancement of the natural agglutinins in the recipients. In a third case, the transfusion was with blood of homologous group and isosensitization had resulted from pregnancy.

A series of 5386 consecutive transfusions in a general hospital transfusion service was reported by DeGowin.[32] Blood of homologous group was employed in practically all cases. Careful tests for grouping and compatibility in the A-B-O system were made. No tests for Rh types or crossmatching for Rh antibodies were performed before transfusion. All clinical transfusion reactions were investigated for evidence of isosensitivity, but most of these tests were made before the blocking antibodies were known. In the entire series there were 186 transfusion reactions of various types, an incidence of 3.4 per cent. In only six of these could isosensitivity to the Rh antigens be proved by the methods then known. Four

recipients had been sensitized by multiple transfusions and two by pregnancy. The incidence of reactions due to isosensitivity was therefore 0.1 per cent. Of the recipients sensitized by transfusion, two were males and two females. But the incidence of reactions of all types in males was 3.1 ± 0.2 per cent in 1112 transfusions as against 3.9 ± 0.4 per cent in 1004 transfusions in females, a significantly greater number in females. The number of maternity patients in the series was small. No recipient manifested clinical signs of isosensitivity with less than four transfusions. There were 399 recipients of both sexes who received four or more transfusions. It was estimated that sixty of these were Rh negative, of which four developed isosensitivity. From these data it was concluded that about 6.6 per cent of the Rh-negative recipients developed isosensitivity to the Rh antigen sufficiently severe to cause transfusion reactions.

Moloney[33] tested eighteen Rh-negative soldiers who had received transfusions of Rh-positive blood and demonstrated the presence of Rh antibodies in the blood of only one. In contrast, Diamond[34] reported the presence of Rh antibodies in the blood of ninety-two of 200 Rh-negative persons who had received transfusions of Rh-positive blood. In twenty-two nulliparous Rh-negative women who had been transfused, he found Rh antibodies in slightly more than one-half. How many of these persons with Rh antibodies would have been unable to tolerate transfusions of antigenic blood was not known. There was a greater incidence of severe reactions when Rh-positive blood was transfused into patients who had been immunized by pregnancy than in those whose isosensitivity had been acquired by blood transfusion.

Number of Transfusions Required to Sensitize. In transfusions of homologous blood groups the majority of the recipients lacking the antigen of the donor probably do not develop acquired antibodies from repeated injections. From a thorough review of the literature Potter[35] concluded that most recipients who became sufficiently immunized to develop clinical manifestations of isosensitivity had required at least three transfusions. Exceptions have been noted, however, in which one transfusion sensitized the recipient to the Rh antigen. Dacie and Mollison[36] reported a case in which Rh antibodies were found in the blood of an Rh-negative recipient after one transfusion of Rh-positive blood. DeGowin[32] studied an Rh-negative woman whose first pregnancy resulted in erythroblastosis fetalis a year after she received a blood transfusion.

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Isosensitization Due to Blood Transfusion

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recipients had been sensitized by multiple transfusions and two by pregnancy. The incidence of reactions due to isosensitivity was therefore 0.1 per cent. Of the recipients sensitized by transfusion, two were males and two females. But the incidence of reactions of all types in males was 3.1 ± 0.2 per cent in 1112 transfusions as against 3.9 ± 0.4 per cent in 1004 transfusions in females, a significantly greater number in females. The number of maternity patients in the series was small. No recipient manifested clinical signs of isosensitivity with less than four transfusions. There were 399 recipients of both sexes who received four or more transfusions. It was estimated that sixty of these were Rh negative, of which four developed isosensitivity. From these data it was concluded that about 6.6 per cent of the Rh-negative recipients developed isosensitivity to the Rh antigen sufficiently severe to cause transfusion reactions.

Moloney[33] tested eighteen Rh-negative soldiers who had received transfusions of Rh-positive blood and demonstrated the presence of Rh antibodies in the blood of only one. In contrast, Diamond[34] reported the presence of Rh antibodies in the blood of ninety-two of 200 Rh-negative persons who had received transfusions of Rh-positive blood. In twenty-two nulliparous Rh-negative women who had been transfused, he found Rh antibodies in slightly more than one-half. How many of these persons with Rh antibodies would have been unable to tolerate transfusions of antigenic blood was not known. There was a greater incidence of severe reactions when Rh-positive blood was transfused into patients who had been immunized by pregnancy than in those whose isosensitivity had been acquired by blood transfusion.

Number of Transfusions Required to Sensitize. In transfusions of homologous blood groups the majority of the recipients lacking the antigen of the donor probably do not develop acquired antibodies from repeated injections. From a thorough review of the literature Potter[35] concluded that most recipients who became sufficiently immunized to develop clinical manifestations of isosensitivity had required at least three transfusions. Exceptions have been noted, however, in which one transfusion sensitized the recipient to the Rh antigen. Dacie and Mollison[36] reported a case in which Rh antibodies were found in the blood of an Rh-negative recipient after one transfusion of Rh-positive blood. DeGowin[32] studied an Rh-negative woman whose first pregnancy resulted in erythroblastosis fetalis a year after she received a blood transfusion.

When the natural agglutinins are enhanced by the transfusion of blood of heterologous group, only one transfusion is required to produce the stimulation.

Time Required to Sensitize by Transfusion. As in other immune reactions a certain time elapses between the injection of the antigen and the development of the antibodies. In the case of the Rh and Hr antigens, at least, this is usually weeks or months. In patients receiving many transfusions Vogel, Rosenthal, and Levine[37] found that sensitivity reactions to further transfusions occurred from twenty-one days to twenty months after the first transfusion. In the case reported by Dacie and Mollison,[38] which seems to be an exception, the Rh antibodies developed so quickly after the first transfusion Rh-positive blood that the transfused red cells were destroyed in the circulation of the recipient in about sixty days instead of the usual 120 days.

In contrast, when blood of heterologous group is transfused, the titer of the natural agglutinins rises rapidly so that the maximum stimulation is often attained by the ninth or tenth day (p. 91).

Transfusion Hemolysis in Isosensitivity

When a person who has developed isosensitivity either by transfusion or during pregnancy, receives a transfusion of antigenic erythrocytes, intravascular hemolysis of the donor's red cells occurs. The extent of the blood destruction and the severity of the clinical manifestations of the reaction probably depend upon the potency of the antibodies of the recipient. It seems plausible to assume that weak antibodies produce a slower rate of hemolysis, so that reactions may be mild or absent. The clinical manifestations of hemolysis and the complications are indistinguishable from those caused by other mechanisms described in Chapter 12. When a recipient of a series of transfusions of homologous group blood exhibits increasingly severe reactions with successive transfusions, the development of isosensitivity should be suspected.

The following cases illustrate several features which have been discussed:

Sensitization by Multiple Transfusions. (Case 38-15984[32]) A man 21 years of age, group O, received ten transfusions of blood of homologous group during the period of a month in the treatment of tuberculosis of the hip. The first four transfusions were without incident. The fifth and sixth were accompanied by chills and fever, but no hemoglobinuria occurred. The seventh and eighth were well tolerated but the ninth and tenth were attended by violent chills and fever At this time, the recipient's blood was tested and the cells were found to be Rh negative The serum contained anti-Rh agglutinins which clumped the erythrocytes of the tenth donor, who was Rh positive Two subsequent transfusions with Rh-negative blood were given without reaction.

Sensitization by Pregnancy Resulting in Transfusion Hemolysis. (Case 44-1090[32]) A woman 49 years of age, group B, had had three normal preg-

nancies resulting in living children. Subsequent pregnancies had produced three miscarriages, one stillbirth, and one infant which had died of umbilical hemorrhage during the second week of life. The last pregnancy had occurred ten years before admission to the hospital for the treatment of chronic cervicitis uteri. She was given two transfusions of group B blood in as many days which were followed by chills, fever, and transient jaundice. Her erythrocytes were found to be Rh negative and the serum contained strong Rh agglutinins. The blood of the last donor was Rh positive. Three subsequent transfusions with Rh-negative blood were given without reaction.

Disappearance of Antibodies After Shocking Transfusion

Several observers have noted that immediately after transfusion of incompatible blood there is a period of several days when the agglutinins of the recipient frequently are lowered in titer or not detectable. Wiener has termed this the *negative phase.* It is attributed to the absorption of agglutinins by the antigen of the transfused erythrocytes. When several days have elapsed the antibodies reappear and can be detected by the usual methods. Thus the evidence of isosensitivity in the recipient may be missed if the serum is only tested immediately after the shocking transfusion.

Prevention of Isosensitization by Transfusion

In the light of recent studies it appears that the most potent source of isosensitization by blood transfusion was eliminated when the practice of grouping and crossmatching of blood in the A-B-O system was introduced. Judged in perspective, additional measures to insure transfusion of blood compatible in the A-B-O system will give diminishing returns, although occasional errors occur in all transfusion services.

There is general agreement that most intragroup transfusion reactions are due to isosensitivity to the Rh antigens in which the recipient has previously been sensitized by the antigen, either through transfusion or during pregnancy. Although intragroup reactions constitute only a small proportion of all transfusion complications (0.1 per cent in DeGowin's series) there is now hope that these may be further reduced by employing the slide technique of Diamond and Abelson (p. 187) in crossmatching to detect the presence of acquired antibodies.

Although transfusion reactions from isosensitivity can theoretically be abolished by using the newer serologic methods, the physician is not relieved of the responsibility for sensitizing the recipient by transfusion. A female child with Rh-negative blood may be sensitized to the Rh antigen through transfusion to such an extent that many years later she may be sterile or bear offspring with

erythroblastosis fetalis in the first pregnancy. *It is increasingly apparent that it is an irresponsible act to transfuse an Rh-negative patient with Rh-positive blood, except in an emergency.*

Precautions which insure against isosensitization to the antigens A, B, and Rh will not, of course, prevent all immune reactions but a great majority will be avoided. The factors M, N, Hr, and O are apparently so poorly antigenic as to require no routine preventive measures.

ISOSENSITIZATION AND PREGNANCY

Hemolytic Disease of the Newborn: Erythroblastosis Fetalis and Related Conditions

History

For many years clinicians had recognized a variety of conditions in the newborn which were regarded as clinical entities. These were designated as *universal edema of the newborn, congenital anemia of the newborn, icterus gravis neonatorum,* and *erythroblastosis fetalis.* In 1932 the studies of Diamond, Blackfan, and Baty[38] demonstrated the essential unity of these disorders. They concluded that all were caused by the same or closely related factors which produced a hemolytic type of anemia in the offspring and that the severity of the process determined the various clinical manifestations which had hitherto been considered separately.

Ottenberg[13] in 1923 and Darrow[39] in 1938 suggested hypotheses which involved antigen-antibody relationships between the blood of the mother and fetus to explain the pathogenesis of erythroblastosis fetalis, but no experimental proof was offered. In 1939 Levine and Stetson[40] reported the presence of an atypical agglutinin in the blood of a woman who had delivered a macerated fetus. The antibody had apparently caused intravascular hemolysis when the patient was transfused with blood of homologous group. The patient's serum agglutinated approximately 80 per cent of a series of bloods of homologous group. The hypothesis was proposed that some factor in the fetus had acted as an antigen which sensitized the mother. In 1940 Landsteiner and Wiener[41] described the Rh factor which was found to be present in the blood of approximately 85 per cent of white persons. In the same year Wiener and Peters[42] reported studies on three patients in whom transfusion reactions were caused by isosensitization to the Rh factor.

In 1940 and 1941 Levine, Katzin, and Burnham[43] suggested that the high incidence of transfusion reactions in pregnant women might be caused by the anti-Rh agglutinins produced in the mother by antigens diffusing through the placenta from the fetus. Statistical

and immunologic studies by Levine and his collaborators,[44] Boorman, Dodd, and Mollison,[45] Mollison,[46] and others have verified the preliminary observations and demonstrated that the great majority of the cases of erythroblastosis fetalis result from isosensitization of the Rh-negative mother by the Rh-positive blood of the fetus.

The occurrence of erythroblastosis in a small number of cases in which the mother and fetus are Rh positive has been explained in some instances by the discovery of another antigenic factor, called the *Hr agglutinogen* by Levine and his coworkers[47] and the *St factor* by Race and Taylor.[48]

Until recently, the lack of evidence of anti-Rh agglutinins in the blood of many sensitized mothers was puzzling. The problem was partially solved by the discovery of Wiener[17] and Race[18] of the blocking or incomplete antibody which masks the action of the agglutinins in tests of cells suspended in saline solution. The development by Diamond and Abelson[21] of the slide method for the demonstration of acquired antibodies has contributed materially to the progress of our clinical knowledge of isosensitization.

Pathogenesis of Erythroblastosis Fetalis

As has been stated previously, the antigen which produces isosensitivity most frequently during pregnancy is the Rh group of agglutinogens. In a small number of cases the Hr′ factor is responsible. In rare instances the A and B substances are apparently antigenic during pregnancy. Sensitization may occur either from the antigens of previous offspring or by injection of antigenic blood during transfusion.

When a woman is Rh negative and her husband is Rh positive, som[illegible]
is [illegible]
hus[illegible]
Wh[illegible]
blo[illegible]
and [illegible] erythroblastosis in the child.

For some reason, not yet clear, more than one pregnancy usually is required to produce isosensitivity in the mother. When the antibodies, both agglutinins and glutinins, are developed in the mother, they diffuse through the placenta into the body tissues and fluids of the fetus and cause hemolytic anemia in the latter. The infant may also obtain antibodies from the mother's milk[49, 50, 51] The rarity with which isosensitization to the A and B agglutinogens occurs in pregnancy, in contrast to the relative frequency of sensitization to the Rh antigens, has been explained by the fact that the A and B substances present in the blood cells of the fetus also

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When a woman is Rh negative and her husband is Rh positive, some or all her children will have Rh-positive blood. If the husband is homozygous, all his offspring will be Rh positive. Should the husband be heterozygous [illegible] negative. [illegible] -positive [illegible] mother and the development of erythroblastosis in the child.

For some reason, not yet clear, more than one pregnancy usually is required to produce isosensitivity in the mother. When the antibodies, both agglutinins and glutinins, are developed in the mother, they diffuse through the placenta into the body tissues and fluids of the fetus and cause hemolytic anemia in the latter. The infant may also obtain antibodies from the mother's milk[49, 50, 51] The rarity with which isosensitization to the A and B agglutinogens occurs in pregnancy, in contrast to the relative frequency of sensitization to the Rh antigens, has been explained by the fact that the A and B substances present in the blood cells of the fetus also

permeate its body tissue and fluid. These substances may then serve as buffers which neutralize the anti-A and anti-B agglutinins which cross the placenta from the mother. Boorman and Dodd[52] have reported, however, that there is a similar distribution of Rh hapten in body fluids.

Although it has not been demonstrated directly, it is assumed[53] that the erythrocytes of the fetus, either intact or disintegrated, cross the placental barrier into the circulation of the mother where they act as antigens. This appears to be a reasonable assumption in the light of the calculation of Dodds[54] that the total length of the placental villi, if placed end to end, is 11.4 miles. Furthermore Hertig[55] and others have observed detached fragments of syncytiotrophoblast in the lungs of a high percentage of women dying in the last months of pregnancy from any cause. Levine[53] has shown that it requires only a small amount of blood to cause sensitization. Agglutinins were produced in rabbits by the injection of human blood in amounts equivalent to 0.0014 to 0.0028 ml. for a woman weighing 120 pounds (60 kg.).

Once the woman has been sensitized to an antigen in the blood of a previous offspring or the blood received in transfusion, her acquired antibodies will induce erythroblastosis in all subsequent offspring whose cells contain the antigen, whereas fetuses without the antigen are unaffected. This has been brilliantly illustrated in the case in which fraternal twins were born to a mother sensitized to the Rh antigen. One of the twins was Rh positive and had erythroblastosis whereas the other twin who was Rh negative was normal.

The usual obstetrical history is that of the Rh-negative woman, with a homozygous Rh-positive husband, who develops sensitization to the Rh antigen during pregnancies. The firstborn is usually normal, and perhaps the second is also. Subsequent offspring have erythroblastosis fetalis in varying degree; usually the severity is progressive with succeeding pregnancies. All the offspring will be Rh positive. If the husband is heterozygous Rh positive, some of the children may be unaffected and they will be found to be Rh negative. Should the firstborn have erythroblastosis, it is probable that the woman was sensitized to the antigen by previous blood transfusion.

Levine estimated that about 92 per cent of cases of erythroblastosis fetalis are due to isosensitization to the Rh antigens. Of these the great majority are caused by Rh_0, while a few are due to rh′ or rh″. About 6 per cent of the total cases are caused by sensitization to the Hr antigens, most of which are Hr′. Rarely is the disorder caused by the A or B antigen present in the blood of the offspring but absent in the mother.

During the pregnancy when the mother is being sensitized to the Rh antigen of the fetus, anti-Rh agglutinins frequently appear in her blood stream in the sixth or seventh lunar month. As the pregnancy progresses the titer of agglutinins increases and blocking antibodies (glutinins) then appear and attain a high titer late in pregnancy. It has been the cause of much conjecture as to why the infants of sensitized mothers are frequently born with few signs of erythroblastosis but the anemia appears and becomes progressive a few hours after birth. Witebsky *et al.*[34] demonstrated that the serum from the cord blood of prematurely born fetuses and full term infants lacks the property of activating the incomplete Rh antibody (glutinin). The activating factor appears in the blood of the infant from twenty-four to forty-eight hours after birth. This suggests that the cause of the explosive development of the anemia in the erythroblastotic infant after birth may be associated with the maturation in the plasma of the factor which activates the glutinins.

Erythroblastosis fetalis occurs with greater frequency in the white race than in any other because of the greater number of Rh-negative persons. The disorder is accordingly rare in the American Negro, the American Indian, the Chinese, and the Japanese.

About 15 per cent of white women are Rh negative and it is estimated that approximately 13 per cent of these marry Rh-positive men. This furnishes the mating which has the potentialities for isosensitization of the mother from the Rh-positive offspring. All statistics agree, however, that only a minority of such marriages produce erythroblastotic offspring. Potter[35] estimated that the incidence of the disease in the Chicago Lying-In Hospital was one in every twenty-two babies born to Rh-negative mothers, or one in about 150 unselected deliveries. These data were obtained at a time when serologic tests were available so that many were diagnosed which would otherwise have recovered unrecognized. The incidence at the Boston Lying-In Hospital was practically the same. Potter has calculated that the Rh-negative woman with an Rh-positive husband has a 1 to 42 chance of having an erythroblastotic offspring in her second pregnancy but the chances increase with each child until in her fifth pregnancy the incidence is 1 to 12.

Clinical Manifestations of Erythroblastosis

Although the various manifestations of hemolytic disease of the newborn are now known to be due to a common cause, Potter[35] has described certain types which are clinically convenient to recognize.

Infants With Late Progressive Anemia. The infants seem well

until one or two weeks after birth when anemia is discovered by noting increasing pallor. Jaundice is usually minimal in this type and the spleen is rarely enlarged. Nucleated red cells are either absent in the blood stream or present in small numbers. Blood transfusions are needed to prevent sudden death. The prognosis in this type is very good for complete recovery. It is probable that many cases in this category are not discovered unless routine serologic tests performed on the mother have demonstrated the presence of the Rh antibodies.

Infants With Progressive Anemia and Jaundice. These appear normal at birth but jaundice becomes evident during the first day of life. Early the erythrocyte count is between 3,500,000 and 4,000,000 per cu. mm. The number of nucleated red cells may not be abnormally large. The spleen may or may not be enlarged. The anemia is progressive and the jaundice deepens for a few days. Transfusions are usually required but the prognosis with treatment is good. When death occurs it is usually between the third and fifth day.

Infants With Severe Anemia and Jaundice. At birth the infant is usually very pale and jaundice is apparent within a few hours. A few ecchymotic spots may be present. The erythrocyte count is usually less than 3,000,000 per cu. mm. The number of nucleated red cells is usually increased on the stained blood film. The spleen is moderately enlarged and there is hepatomegaly. The child is very lethargic. Prompt transfusion results in survival of some of these children, but many injections of blood are usually necessary. The icterus may persist for weeks. The face and extremities may be slightly edematous and the placenta is hypertrophic.

Infants With Generalized Edema. There is little or much edema, although the skin is not involved. The pleural and abdominal cavities may contain much fluid. The erythrocyte count is frequently less than 1,000,000 per cu. mm. A great proportion of the nucleated cells are immature erythrocytes. The liver and spleen are enlarged. There is usually no icterus. The placenta is edematous and its weight is greatly in excess of normal. The child either dies during delivery or a few minutes later. Treatment is of no avail.

Pathology of Erythroblastosis

The fetuses which die during the first few months of pregnancy rarely show any of the typical findings of erythroblastosis fetalis. When the offspring succumbs *in utero* within a few weeks of expected delivery, the skin is usually macerated and frequently the face is edematous and the tongue protrudes. The spleen is invariably enlarged and there may be moderate enlargement of the liver. Evidence of increased extramedullary hematopoiesis is obtained by

histologic examination. The heart is slightly hypertrophied. If the infant was born alive, there is usually marked icterus of all tissues and fluids. There are depositions of bile pigment in the basal nuclei and other parts of the brain, together with pigmentation of the renal pyramids and focal necrosis of the white matter of the brain. The pigmentation of the basal nuclei is known by the German term *kernicterus* and is considered by Gilmour[17] as pathognomonic of erythroblastic disease.

Blood Counts in Erythroblastosis

It is usually stated that nucleated red cells are in normal concentration in the newborn when they constitute less than 10 per cent of the cells containing nuclei, as counted on the stained blood film. This is predicated upon the leukocyte count being about 10,000 per cu. mm. Since the leukocyte count may vary considerably, it is more accurate to count the proportion of nucleated red cells to leukocytes on the film, estimate the number of leukocytes in the counting chamber, and compute the number of nucleated erythrocytes per cubic millimeter. It is therefore more exact to state that the upper limit of normal for nucleated red cells is 1000 per cu. mm. In most cases of erythroblastosis there is an initial excess of nucleated red cells, but in the milder cases these disappear rapidly from the circulation so that counts a few days after birth are normal.

The total erythrocyte count in hemolytic disease is rarely over 5,000,000 per cu. mm. and, in the severe forms, it may be less than 1,000,000. The plasma proteins are usually reduced in the edematous forms of the disorder.

Clinical Course of Erythroblastosis

Most deaths from the disease occur within the first day of life. The prognosis for survival is good after the third day. In the milder grades of the disorder the infants acts normally. Lethargy is common in the presence of extreme icterus. The food may be regurgitated and many loose stools are passed. The feces may be nearly completely acholic for a short time. The jaundice usually reaches its maximum by the end of the first week and then the bilirubin in the skin is converted to biliverdin, imparting a distinctly green color to the tissues. The degree of pigment infiltration of the brain is not always proportional to the intensity of the jaundice elsewhere. Signs of kernicterus are lethargy, hypertonicity, opisthotonos, and convulsions. The usual course in hemolytic disease, however, is gradual improvement after the first few days. Blood transfusions may be required for weeks until the rate of erythrocyte destruction lessens.

TREATMENT OF ERYTHROBLASTOSIS

As in the hemolytic anemias of adults, the principal treatment consists in the transfusion of blood. At present there are divergent opinions as to the most efficacious manner in which blood can be administered. In the evaluation of therapy in this disorder it should be remembered that spontaneous recovery without treatment is the rule in the milder cases. Final judgment as to the efficacy of treatment can only be made by careful study of large numbers of patients. Since the disorder is relatively uncommon, it follows that only those obstetricians who practice in large maternity clinics where careful serologic tests are made routinely are equipped to make the necessary clinical evaluation.

Type of Donor's Blood. The tissues and fluids of the erythroblastotic infant are saturated with maternal antibodies which are incompatible with the infant's erythrocytes. Mollison[67a] has clearly shown that transfused Rh-negative red cells survive in the circulation of the Rh-positive infant with hemolytic disease much longer than do Rh-positive cells. In this peculiar transfusion situation cell survival depends upon compatibility of the donor's erythrocytes with the antibodies of the mother rather than those of the recipient. This is because the newborn infant has no antibodies of its own. For this reason Wiener and Wexler[68] suggested that in the absence of a suitable donor the washed red cells of the mother could be transfused. There seems to be little doubt that the amount of destruction of *donor's blood* can be minimized by transfusing erythrocytes which are not acted upon by the maternal antibodies in the tissues of the infant. This consideration may be important in attempting to avoid the remote and permanent damage from kernicterus.

Contrariwise, there are those who advocate the transfusion of Rh-positive blood into the infant saturated with Rh antibodies. Two arguments are advanced. The difference in survival time between Rh-negative and Rh-positive red cells is not thought to be clinically significant. Transfused Rh-positive cells should fix some of the harmful antibodies in the circulation of the infant Theoretically, this should minimize the destruction of the *recipient's blood.* Sufficient clinical data are not available to enable a final choice between the two courses of treatment although it is believed that the preponderance of opinion favors the use of Rh-negative blood in transfusion.

Repeated Small Transfusions. The natural tendency has been to transfuse citrated blood in 50-ml amounts into the infant whenever the hemoglobin concentration or the erythrocyte count

falls below a value which is considered desirable. During the first day of life the transfusion may be given through a catheter inserted in the umbilical vein. This vessel soon thromboses, however, and injections must thereafter be made through a needle into peripheral veins. The method of repeated small transfusions possesses two disadvantages. Frequently a large number of venipunctures must be made in very small vessels. Secondly, the method makes no attempt to arrest the progress of hemolysis of the infant's blood, so that kernicterus is probably not altered by treatment. Witebsky *et al.*[16] demonstrated that the admixture of even small amounts of normal adult plasma with the plasma of the newborn activates the glutinins on the infant's erythrocytes so that agglutination occurs in the test tube. It was estimated that the injection of 50 ml. of adult normal blood into the infant's circulation might cause a similar effect, although direct proof is not yet available.

Exchange (Substitution or Exsanguination) Transfusions. Wallerstein[59] devised a method of exchange or substitution transfusions for the treatment of erythroblastosis. In the *intermittent type* 50 ml. of Rh-negative blood is injected into the saphenous or other convenient vein, and then a corresponding amount is withdrawn. If the infant's blood volume is 250 ml., five such exchanges will result in the substitution of approximately 75 per cent of its blood. A *continuous procedure* was also devised in which blood is injected into the saphenous vein and withdrawn at the same time through the superior sagittal sinus. If this procedure is carried out for sixty minutes it is estimated that only 36.7 per cent of the infant's blood will remain. Wiener *et al.*[60] have modified this technique by giving a continuous transfusion of 500 ml. of blood into the ankle vein and withdrawing blood nearly as fast through a nick in the radial artery on the other side. Doses of 200 units of heparin are injected into the vein at the commencement, the first quarter, and at the middle of the transfusion. The volume of injected blood is kept at about 50 ml. in excess of that withdrawn. This procedure is said to exchange 90 per cent of the infant's blood. The authors claim no untoward hemorrhages from the heparin. Platou *et al.*[61] have accomplished exchange transfusion by the intermittent method through a catheter in the umbilical vein.

The advantage of exchange transfusion is that most of the infant's erythrocytes are removed and replaced by red cells which have a normal survival expectancy so that the hemolytic process is arrested. Frequently no further transfusions are required.

Weaning the Baby. It is thought that the infant should not use the milk from the mother. Witebsky *et al.*[50,51] have demon-

strated the presence of Rh agglutinins and glutinins in the milk of the sensitized mother.

Interruption of Pregnancy. Some clinicians have advocated the interruption of pregnancy in the last few weeks before term when the serologic tests indicate the probability that the offspring is developing erythroblastosis. This policy is based on the observation that antibodies in the mother's blood increase greatly during the last few weeks of the pregnancy and that the newborn infant seems to develop the symptoms of hemolytic disease some hours after birth. For these reasons cesarian section has been proposed and practiced. No conclusive evidence has yet been presented to show that the mortality from erythroblastosis fetalis has been reduced by this procedure whereas it is obvious that the infant is exposed to the mortality from the operation plus the handicap of prematurity.

SEROLOGIC FINDINGS IN THE MOTHER

Blood Group and Type of the Mother. As has already been stated, about 92 per cent of the women giving birth to erythroblastotic offspring belong to the types rh, rh′, rh″, or rh′rh″ and become sensitized to the Rh_0 antigen in the blood of the fetus. To furnish the Rh_0 antigen to the mother, the blood of the fetus must belong to types Rh_0, Rh_1, Rh_2, or Rh_1Rh_2. Rarely is a case encountered in which the woman is sensitized only to the rh′ or rh″ antigen.

About 6 per cent of the sensitized women belong to subtypes Rh_1Rh_1 or rh′rh′ and become immunized to the Hr′ antigen which their erythrocytes lack. Extremely rare is the case of sensitization to the Hr_0 or Hr″ antigen.

A few cases have been reported in which the A or B agglutinogen in the erythrocytes of the fetus have been considered as the antigens which sensitized the mother who lacked these substances in her own blood. The proof for this has been (*a*) incompatible blood groups in mother and child in the A-B-O system, (*b*) the lack of anti-Rh or anti-Hr antibodies in the mother's serum, and (*c*) the presence of an unusually high titer of the appropriate anti-A or anti-B agglutinins in the mother's serum.

Antibodies in the Mother's Plasma. The acquired agglutinins (*complete, bivalent, or early immune antibodies*) *frequently appear* in the blood of the mother during the fifth or sixth lunar month, or later. If the blood serum is titrated monthly thereafter, progressive increases in potency are found. One or two months after the appearance of the agglutinins the presence of glutinins (blocking, incomplete, hyperimmune, univalent, or inhibitor antibodies)

may be detected. These in turn may increase during the remainder of the pregnancy. A further increment in the titer of antibodies is frequently observed seven to twenty-one days after delivery.[45] Both orders of acquired antibodies usually have disappeared from the circulation five or six months after delivery, although occasionally they have been found persisting five to eight years after gestation. The disappearance of antibodies from the circulation does not indicate loss of sensitivity because contact with the specific antigen quickly stimulates their reappearance.

Glutinins are usually specific for the Rh_0 antigen, although this is not invariably true. When sensitization occurs to fetal cells of the types Rh_1 or Rh_2 it is frequent to find that the mother's serum contains glutinins for type Rh_0 and agglutinins for Rh_0 and rh′ or rh″. When such sera are tested with erythrocytes suspended in serum they have the specificity anti-Rh_0′ or anti-Rh_0″, but because of the blocking antibodies they may act as anti-rh′ or anti-rh″ sera against cells suspended in saline solution.

The progressive increase in titer of Rh or Hr antibodies in the serum of the mother more than ten weeks before delivery usually indicates developing isosensitization and consequent erythroblastosis in the fetus.[62] This is not, however, invariably true because instances of anamnestic reactions are occasionally observed.

Anamnestic Reactions. Occasionally a woman who has been previously sensitized to the Rh antigen, either by pregnancy or transfusion, will exhibit the usual increase in titer of antibodies during a succeeding pregnancy, only to deliver a normal infant who is Rh negative. Presumably this is an anamnestic reaction in which the antibodies are stimulated by some nonspecific factor in the pregnancy.

In a group of twenty-two mothers in which Rh antibodies were found more than ten weeks before delivery Page, Hunt, and Lucia[62] reported the occurrence of sixteen offspring with erythroblastosis fetalis and six normal infants. Four of the normal babies were Rh negative and came from mothers previously sensitized, but the absence of hemolytic disease could not be explained in the other two.

Antibodies in Milk and Colostrum. Acquired antibodies (agglutinins and glutinins) are found in the breast milk and colostrum of most sensitized women.[50,51] The titer is approximately equal to that in the blood serum.

Serologic Findings in the Infant

The erythrocytes of the infant contain the antigen which sensitized the mother. The antibodies of the mother, especially the

strated the presence of Rh agglutinins and glutinins in the milk of the sensitized mother.

Interruption of Pregnancy. Some clinicians have advocated the interruption of pregnancy in the last few weeks before term when the serologic tests indicate the probability that the offspring is developing erythroblastosis. This policy is based on the observation that antibodies in the mother's blood increase greatly during the last few weeks of the pregnancy and that the newborn infant seems to develop the symptoms of hemolytic disease some hours after birth. For these reasons cesarian section has been proposed and practiced. No conclusive evidence has yet been presented to show that the mortality from erythroblastosis fetalis has been reduced by this procedure whereas it is obvious that the infant is exposed to the mortality from the operation plus the handicap of prematurity.

Serologic Findings in the Mother

Blood Group and Type of the Mother. As has already been stated, about 92 per cent of the women giving birth to erythroblastotic offspring belong to the types rh, rh′, rh″, or rh′rh″ and become sensitized to the Rh_0 antigen in the blood of the fetus. To furnish the Rh_0 antigen to the mother, the blood of the fetus must belong to types Rh_0, Rh_1, Rh_2, or Rh_1Rh_2. Rarely is a case encountered in which the woman is sensitized only to the rh′ or rh″ antigen.

About 6 per cent of the sensitized women belong to subtypes Rh_1Rh_1 or rh′rh′ and become immunized to the Hr′ antigen which their erythrocytes lack. Extremely rare is the case of sensitization to the Hr_0 or Hr″ antigen.

A few cases have been reported in which the A or B agglutinogen in the erythrocytes of the fetus have been considered as the antigens which sensitized the mother who lacked these substances in her own blood. The proof for this has been (*a*) incompatible blood groups in mother and child in the A-B-O system, (*b*) the lack of anti-Rh or anti-Hr antibodies in the mother's serum, and (*c*) the presence of an unusually high titer of the appropriate anti-A or anti-B agglutinins in the mother's serum.

Antibodies in the Mother's Plasma. The acquired agglutinins (complete, bivalent, or early immune antibodies) frequently appear in the blood of the mother during the fifth or sixth lunar month, or later. If the blood serum is titrated monthly thereafter, progressive increases in potency are found. One or two months after the appearance of the agglutinins the presence of glutinins (blocking, incomplete, hyperimmune, univalent, or inhibitor antibodies)

glutinins, can frequently be demonstrated in the infant's serum in titers almost as high as in the serum of the mother.

The glutinins from the mother which are adsorbed on the infant's erythrocytes can be demonstrated in several ways. The washed erythrocytes are agglutinated by anti-human globulin serum if they have adsorbed acquired antibodies (see *Coombs' Test*, p. 176). They are likewise agglutinated when normal adult neutral serum is added (see *Witebsky's Test*[16]). Sometimes these tests on the infant's cells indicate isosensitivity when no antibodies can be demonstrated by other means in the mother's serum.[28]

Prognosis in Subsequent Pregnancies

There is general agreement that once a woman has been sensitized *to an antigen and produced an erythroblastotic fetus, all* subsequent offspring with the antigenic red cells will meet the same fate.

The physician is frequently consulted about the prognosis for future pregnancies in the family in which erythroblastosis has occurred. If the woman is sensitized to the Rh antigen, a partial answer may be obtained in many cases by determining whether the husband's Rh genotype is homozygous or heterozygous. Two lines of evidence may be sought.

(A) If there are surviving children from the mating, their Rh types should be determined. Should one prove to belong to type rh, this is proof that the husband's genotype contains the gene *r* (cde), always assuming that the paternity of the child is as represented. The fact that none of the children are Rh-negative proves nothing as to the genotype of their father.

(B) Should the husband belong to type Rh_1 or rh′, the reaction of his erythrocytes to anti-Hr′ (anti-c) serum should be tested. Agglutination by this reagent indicates that he belongs to subtypes Rh′rh or Rh_1rh, as the case may be, and that 50 per cent of his sperm contain the gene *r* (cde). There is theoretically an even chance in any succeeding pregnancy that the offspring will have the genotype *rr* (cde/cde), i.e., Rh negative, and therefore escape having erythroblastosis.

Prevention of Erythroblastosis Fetalis

Women and female children who are Rh negative should not receive transfusions of Rh-positive blood, except in emergencies. The evidence is quite clear that permanent isosensitization can occur from transfusions of blood containing the antigen which were given many years before. Once sensitization has occurred there is no known method of desensitization.

Artificial insemination of women sensitized to the antigen in their husband's blood has been successfully performed with the sperm from Rh-negative males.[63]

As a generality, advising Rh-negative women against marriage with Rh-positive males is scarcely practical, especially since only one in twenty will become sensitized to the Rh antigen.

Some state departments of health routinely determine and report the Rh type of all blood specimens which are required by law to be submitted for serologic tests for syphilis before marriage. Apparently the purpose is to acquaint the prospective parties to a marriage of the fact that the woman is Rh negative and the man Rh positive. It is difficult to understand how this serves a useful purpose. There is no question that it causes much unnecessary anxiety on the part of the individuals involved. Frequently there is grave misunderstanding as to the significance of the results of the tests. It would seem much more desirable for the physician who attends the woman during pregnancy to obtain the Rh typings and explain the significance to the patient.

Remote Sequelae of Erythroblastosis

Undoubtedly some of the infants who survive severe degrees of hemolytic disease of the newborn suffer permanent damage from the deposition of bile pigment and the anemia which occur at the critical stage of development. At present, however, the reports of permanent damage are fragmentary and circumstantial and most of the data are insufficient to be statistically significant. Further studies are needed.

Central Nervous System. Several attempts have been made to demonstrate a significant association between erythroblastosis and undifferentiated mental deficiency. Yannet and Lieberman[64] and others have found a slightly increased incidence of Rh-negative mothers of Rh-positive children with feeblemindedness. This seems to have been substantiated. The exact significance of this finding is not yet clear. It is, of course, possible that the deposition of pigment in the brain during hemolysis might cause permanent damage.

It seems more firmly established that the infant who survives severe erythroblastosis with kernicterus may have permanent impairment of motor function in the form of hypotonia, muscular rigidity, athetosis, and diplegia.

Liver. Apparently the liver is sometimes damaged in erythroblastosis fetalis. Henderson[65] reported finding evidence of hepatic cirrhosis in three macerated fetuses which had died from erythroblastic disease. Drummond and Watkins[66] studied three families,

the children of which had had hemolytic disease of the newborn. Several children were found to have enlarged livers, and in some cases large spleens, at ages ranging from seven to thirteen years.

Skeletal System. In a study of forty-seven cases of erythroblastosis fetalis, Javert[67] noted that congenital malformations were about forty times as common as in a series without erythroblastic disease. The types of disturbances were harelip, cleft palate, spina bifida, cervical rib, supernumerary digits, hydrocele, urethral stricture, and defect of the intraventricular septum of the heart. Wiener[68] studied nineteen families in which congenital malformations, particularly spina bifida and hydrocephalus, were frequent in the offspring. In each case either the mother's serum contained abnormal acquired antibodies for the Rh-Hr antigens or the maternal anti-A or anti-B agglutinins, which were specific for antigens in the offspring's cells, were much more potent than normal. This suggests that isosensitization in the mother has some causal relationship to the malformations in the fetus.

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CHAPTER 9

Laboratory Procedures

By Elmer L. DeGowin

GENERAL CONSIDERATIONS

Grave Responsibility of the Technician

The serologic examination of the blood for antibodies and agglutinogens before transfusion places a responsibility upon the technician which is almost unique in clinical medicine. Practically all other tests performed in the laboratory of the hospital are diagnostic in nature and the clinician employs the results merely as adjuncts to the information obtained from other sources, such as the history of the illness and the physical findings in the patient. Thus the physician is enabled many times to suspect errors which occur in the laboratory when the reported results are incongruous with the clinical findings. Furthermore, it is rare that a patient is subjected to a procedure which is hazardous to life solely because of the findings in a urinalysis or a blood cell count. But when the technician performs compatibility tests before blood transfusion, his or her responsibility for the welfare of the patient is vastly increased. An error in making or interpreting the tests may directly result in subjecting the patient to a dangerous or fatal reaction from blood transfusion.

Serologic tests on blood are peculiarly vulnerable to error. The reasons for this are: (1) There are few inherent checks in the tests themselves. (2) A negative reading has equal or more weight than a positive result. The absence of agglutination is accepted as evidence in blood grouping and is used as proof of compatibility in crossmatching. (3) On some occasions the tests before transfusion are performed under conditions of extreme urgency and hence the technician is placed under the psychological handicap of working against time. These considerations force the following conclusions: The technician who is entrusted with the laboratory tests related to blood transfusion must have a thorough training and understanding of the procedures. The tests have become much more complicated in the last five years so that it is desirable that the technician have personal instruction from one entirely familiar with the field. Every precaution should be taken to permit the technician opportunity to perform adequately the proper tests.

Isohemagglutination

Stages of Agglutination. The phenomena of agglutination occur in two parts. During the *First Stage*, or the phase of antigen-antibody combination, the agglutinins in the serum or plasma combine with the specific agglutinogens in the red cells. The charge on the surface of the cells is thought to be altered so that erythrocytes similarly treated adhere when brought together. The *Second Stage*, or the phase of clumping, is that during which the altered cells make contact and form agglutinates.

Natural and Acquired Agglutinins. There are important differences in the properties of isohemagglutinins which appear to be related to their origin, i.e., whether they occur naturally, or as a result of isosensitization. These differences may be summarized as follows:

	Natural Agglutinins	*Acquired Agglutinins*
Designations	Anti-A; Anti-B	Anti-Rh, Anti-Hr
Optimal temperature for antigen-antibody combination	±5° C.	37° C.
Combination time of antigen and antibody	immediate	delayed
Cohesion of agglutinates	strong	weak
Inactivation by dilution of protein	slight	great
Blocking antibodies.	not present	frequently present
Isohemolysins	present in 30%	not present

Effect of Temperature. The temperature range from 2° to 50° C. will be considered. In general, the natural agglutinins anti-A and anti-B combine with agglutinogens slightly better at low

temperatures than at room or body temperature. The differences, however, are not sufficiently great to warrant special attempts to deviate from room temperature in performing routine tests for blood grouping and crossmatching in the A-B-O system. The titer of most natural agglutinins falls off rapidly as the temperature is increased from 37° to 50° C. The acquired (immune) agglutinins, such as the anti-Rh and anti-Hr antibodies, generally have their optimal reaction at 37° C. Most cold hemagglutinins are active only at about 5° C. and are not demonstrable at room or body temperature. If the titer is high, however, they may be effective enough to interfere with blood grouping or crossmatching at the temperatures at which those tests are usually performed.

Acceleration by Agitation. Although the antigen-antibody union is nearly instantaneous in the A-B-O system, the time taken for the altered cells to make mutual contact is extremely variable, depending on the method employed in the test. If a mixture of specific serum and erythrocyte suspension is permitted to stand in a test tube, the maximum number of contacts between cells is not attained until the red cells have fallen to the bottom. When the depth of the fluid is several millimeters, the complete sedimentation necessary for agglutination may not occur until sixty to 120 minutes. On the contrary, if the cell-serum mixture is placed on a glass slide, the fluid layer is only a fraction of a millimeter in depth and sedimentation occurs in fifteen to thirty minutes. Any maneuver which hastens contact between cells accelerates agglutination. If the mixture on the slide is continuously agitated by rotation, tilting, or shaking, clumping is usually complete in ten to fifteen minutes. Centrifugation of the test tube for one minute at 500 to 1000 revolutions per minute results in complete agglutination. Cognizance of these facts materially shortens the time consumed in blood grouping and crossmatching.

The principle of acceleration of agglutination applies likewise when the anti-Rh and anti-Hr antibodies are concerned, but sufficient time must be allowed for the first stage which is much slower than in the A-B-O system.

Types of Acquired Antibodies. Early in the course of isosensitization of the individual specific agglutinins may be formed. These are termed *early* or *immune* (Diamond), *bivalent* (Wiener), or *complete* (Race). Their characteristic feature is that they produce agglutination when cells of suitable type are suspended in isotonic saline solution.

As the isosensitization of the individual becomes more intense another type of antibody may be formed. This has been designated as *late*, *mature* or *inhibitor* (Diamond), *blocking* or *univalent* (Wiener),

incomplete (Race). It is distinguished from the simple agglutinin by the fact that it inhibits the action of agglutinins of like specificity when the test cells are suspended in isotonic saline solution. When the erythrocytes are suspended in protein solutions to which little or no electrolyte has been added, these antibodies cause agglutination. This fact is illustrated by the experiment in which blocking antibody is added to suitably specific erythrocytes which are suspended in saline solution and incubated at 37° C. for sixty minutes. No clumping of the cells is observed after such a procedure. If the supernatant saline is withdrawn and replaced by inert serum or albumin, agglutination then occurs. There can be little doubt that blocking antibodies act as agglutinins *in vivo* and that their differentiation from the simple agglutinins *in vitro* is but a fortuitous circumstance. Wiener terms the two antibodies *agglutinins* and *glutinins* and maintains that the cell clumping caused by the latter is different from true agglutination.

Differentiation of Acquired Antibodies. In testing sera for evidence of isosensitivity four possible situations may be encountered: (1) no antibodies are present; (2) only acquired agglutinins are demonstrable (early isosensitivity); (3) only blocking antibodies are encountered (late isosensitivity); (4) a mixture of agglutinins and blocking antibodies is found (late isosensitivity).

When the unknown serum is incubated with erythrocytes suspended in inert plasma, serum, or albumin, and no agglutination results, the presence of both agglutinins and blocking antibodies has been excluded. If agglutination results in this test, the action of the serum should be tested against the same cells suspended in isotonic saline solution. Parallel titrations of the serum against cells suspended by the two methods may reveal whether there are the two types of antibodies present or which type, if only one, is concerned.

Erythrocyte Suspensions. The satisfactory performance of any technique involving isohemagglutination cannot be hoped for unless the prescribed concentration of red cells is employed. The nature of the diluent used in the suspension of the test cells must be correct. Most agglutination tests are performed with a 2 per cent concentration of red cells in the suspension. If the concentration is much less, the tests are difficult to read and the time required for agglutination is prolonged. If the concentration greatly exceeds the optimum, the agglutinogens may completely absorb the anti-A and anti-B agglutinins from weak sera so that clumping does not occur when expected.

A 2 per cent cell suspension may be approximated by adding a full drop of whole blood to about 1 ml. of diluent. With a little

practice the technician may be able to judge the correct concentration by the color and turbidity of the suspension.

Until recently erythrocyte suspensions were made in isotonic saline solution (0.9 per cent NaCl). It has been found, however, that a hypertonic solution of 1.4 per cent sodium chloride gives optimal agglutination, both in the size of the clumps and in the speed of the reaction. Hence this concentration may be recommended when saline is indicated as a diluent for cell suspensions in agglutination tests.

In certain tests cell suspensions are desired in which no electrolyte is employed in the diluent. The erythrocytes may then be suspended in human serum, plasma, or serum albumin, or bovine albumin. The human serum should contain no interfering agglutinins. The cells may be suspended in their own serum or plasma, or in that derived from a person who belongs to group AB.

Testing With Weak Agglutinins. In blood grouping or typing, sera are selected which have high titer to insure prompt and complete reactions. In crossmatching, however, there is no choice but to work with agglutinins which are frequently of indifferent potency. Centrifugation assists the reaction by securing maximum contacts of sensitized cells. In addition, it has been shown that the action of weak agglutinins is accentuated by increasing the proportion of serum to erythrocytes. Instead of the usual equal parts of serum and cell suspension, one part of a 1 per cent cell suspension may be used with ten parts of serum. Higher titers of agglutinins are attained by this procedure.

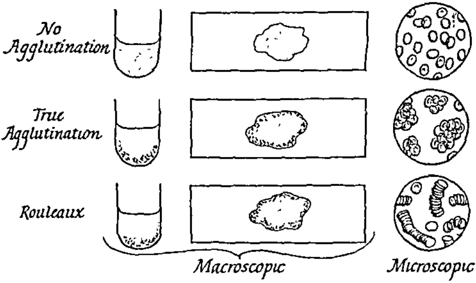
Perception of Agglutination. Considerable practice is required to determine the presence or absence of agglutination by any method of examination. The inexperienced observer has a decided tendency to mistrust the evidence obtained with the unaided eye and to rely too heavily upon the microscope. Microscopic examination has many pitfalls, the chief of which is mistaking other phenomena for agglutination. The chance contact of several erythrocytes, clumps of fibrin or debris, or the occasional agglomeration of cells seen in stored blood are erroneously concluded to be evidence of agglutination. The microscope should be reserved for the occasional doubtful case, for the differentiation between true agglutination and rouleaux, and for the identification of the small agglutinates caused by weak anti-Rh and anti-Hr sera.

The serum-cell mixture may be inspected macroscopically on a glass slide with proper lighting. Large clumps of red cells are seen easily against a background of white paper or tile several inches below the slide. Smaller clumps may be discerned when a narrow slit of light is permitted to pierce the slide perpendicularly

from below, and the line of vision impinges upon the mixture at an acute angle so that light does not shine directly into the eyes of the observer.

Mixtures in tubes are more difficult to illuminate properly. A convenient method is to place the substage mirror of a microscope on the table with the concave side up. The tube is held almost horizontally about 2 to 3 cm. above the mirror. The observer looks at the *reflection* in the mirror which magnifies the image slightly. The tube may be illuminated by a desk lamp shining on the tube, but not into the eyes of the observer. If small tubes are employed, they may be placed horizontally on the stage of the microscope and examined with the low power objective.

APPEARANCE OF ISOHEMAGGLUTINATION



In examining agglutinates produced by anti-Rh or anti-Hr agglutinins or blocking antibodies, special precautions must be observed in handling the serum-cell mixtures. The cells in the clumps adhere but slightly and are easily separated by agitation. The preferred method of inspection is to remove the tube or slide carefully and place it over the site of illumination. The tube is then turned slowly to the horizontal position and rotated once or twice, part way. During the manipulation the cell mass is observed as the parts separate, and an estimate is made as to whether the parts are larger than in a control suspension in which no agglutination is present. If slides are employed, they are slowly tilted back and forth. The interpretation takes considerable practice.

Isohemolysis

About 30 per cent of sera which contain the anti-A or anti-B agglutinins also have isohemolysins of corresponding specificity. No hemolysins have been recognized in association with anti-Rh or anti-Hr agglutinins. The hemolysins have a lower titer than the corresponding agglutinins. When a serum containing both agglutinins and hemolysins is added to red cells with receptive agglutinogens, agglutination occurs first and the cell clumps partly or completely disappear later. If the reaction occurs in a tube with sufficient depth of cell suspension, the appearance of the fluid changes from the pink, opalescent gray color of suspended erythrocytes to a clear red, transparent solution. When small amounts of serum-cell mixture are employed, however, as in the case of many of the tests described in this chapter, gross hemolysis is difficult to discern. Microscopic observation is of no great value in this situation. There are always some inagglutinable erythrocytes to be seen which may be mistaken for the entire number with which the test was begun.

In general, the higher the titer of agglutinins, the more likely the occurrence of associated isohemolysins. The agglutinates formed in the presence of hemolysins are usually smaller than otherwise. Isohemolysis occurs only when fresh serum or plasma is employed because the phenomenon depends on the presence of the labile *complement*, which is inactivated by aging at room temperature for two or three days, storage at 2° to 5° C. for two or three weeks, or exposure at 56° C for thirty to sixty minutes. Complement may be preserved for long periods, however, in serum or plasma which is kept in the frozen or dry state. Dilution of the fresh serum four times with isotonic saline solution usually will inactivate it.

Grouping sera are treated to inactivate the complement so that hemolysis is not encountered in blood grouping. In crossmatching, however, the complement in the serum or plasma of the prospective recipient is not customarily destroyed and is a source of particularly dangerous error. *Prompt reading of tests for agglutination, before hemolysis occurs, will prevent some errors due to the action of isohemolysins.*

Potency of Antisera

The concentration of agglutinins, hemolysins, and blocking antibodies can be judged approximately by titration or by measuring the avidity time.

Titration of Sera. In this procedure measured dilutions of the unknown serum are tested for their ability to react against suspen-

sions of suitable erythrocytes. The tests usually are performed in serologic test tubes or upon well slides.

Serum Dilutions. It is customary to make dilutions of serum which increase by geometric progression, that is, each succeeding tube contains twice the dilution of the preceding one. Occasionally this plan is altered when the potency of the serum is low or a more exact reading is desired. The dilutions are made in small tubes, regardless of whether the final tests are to be performed in the tubes or on well slides. For the most exact measurement, each transfer of serum should be made with a separate clean, dry serologic pipette. This procedure uses a large number of pipettes and the slight increase in accuracy probably does not justify the trouble for ordinary purposes. An alternate method is to employ the same pipette throughout the entire series of dilutions, washing it in saline solution between each transfer. An ungraduated pipette with a capillary tip can be employed by delivering the same number of drops with each transfer.

In expressing serum dilutions no correction should be made for the volume of cell suspension which is added to the serum in the test. The original diluting of the serum should be regarded as a means of counting the number of antibodies which are placed in each tube. This number usually is in excess of that required to cause agglutination so that several times the volume of cell suspension could be agglutinated.

Serum Titer and End Point. The greatest dilution of serum in which agglutination is discernible is taken as the end point of the reaction. The reciprocal of the end-point dilution of the serum is the *titer.* If the greatest dilution of serum showing agglutination, for example, is 1/128, then the titer is 128. There are several criteria for reading the end point in agglutination reactions. The present authors prefer reading the tests by macroscopic observation. This gives the lowest titer but the readings are the most clear-cut. Furthermore, grouping sera usually are employed in tests in which macroscopic readings are relied upon and this criterion seems the most logical. Reading the end point with a hand lens or microscope results in higher titers but the results frequently are not sharp.

Methods of Agitation. There are several methods by which the sensitized cells in a titration procedure may be brought into mutual contact during the second stage. These are discussed on page 121. In general the centrifuge technique is recommended as being faster and giving sharper readings.

Avidity. This is defined as the speed with which an agglutinin serum causes agglutination of red cells which contain the specific agglutinogens. The procedure must be defined with exactness

because slight variations will cause differences in the reaction time. In general, there is good correlation between the titer of an agglutinin and its avidity, and the higher the titer the quicker agglutination occurs. Occasionally, however, a serum is encountered which acts slowly although cells are agglutinated by high dilutions. These exceptions have not been adequately explained. The avidity of a serum seems to be increased when the test erythrocytes are suspended in hypertonic saline solution (1.4 per cent) instead of an isotonic concentration. Tests for avidity are quicker to perform than titrations of serum in test tubes. We are not convinced that they are any more accurate. Perhaps both types of tests should be performed in evaluating the potency of an agglutinin serum.

Variations in Observed Titer and Avidity. There is a tendency to regard relationships expressed in mathematical terms, such as titer and avidity, as being especially accurate and reproducible. It is possible for two workers who employ the same serum and the same suspension of red cells to approximate closely the values obtained by each for titer and avidity time. Both, however, must follow precisely the same method and observe identical criteria for the end point of the reaction. A variation of one dilution in reading the titer is permissible because the same person cannot repeat the test with greater accuracy. But numerical titers and avidity values have little significance except to the worker who determines them. When presenting data to others, they are admissible as evidence only when compared with other data by the same worker. In a wartime project eleven trained observers,[1] each using his own method, with different criteria for the end point of reaction, and test cells from different sources, attempted to evaluate the same anti-A and anti-B sera. The titer of a serum was reported by one observer as 1/32, by another as 1/1000. In another case the avidity of a serum was recorded by one as fifteen seconds and by another as sixty seconds. The variations between these extremes resulted not only from differences in technique but possibly from gradations in the sensitivity of the erythrocytes from diverse sources. Yet there was good agreement among the workers as to which sera were potent and which were not.

Standard Reference Sera. As a result of this variability in results, standard reference sera for anti-A and anti-B agglutinins were prepared in stable form (dry). The sera were accepted arbitrarily because they were judged to be potent by a number of observers, using their own criteria. No attempt was made to select sera of maximum or minimum potency. The standard serum was tested in parallel with the unknown serum, using the same procedure and the same erythrocyte suspensions. Thus the strength of the

unknown serum could be compared directly with the reference serum, and its potency expressed in terms of the strength of the reference serum. The symbol R was employed to denote that the serum had approximately the same strength as the reference serum, 2R for twice the strength, and 0.5R for half the potency. Reference sera are now available for qualified workers at the National Institute of Health.

Clotting of Serum-Cell Mixtures

In serologic tests serum is sometimes added to citrated plasma. Frequently this combination results in clotting, so that agglutination reactions cannot be properly observed. Coagulation occurs because there is sufficient thrombin in the serum to react with the fibrinogen in citrated plasma to form fibrin. The calcium ion in the plasma assists in the union of thrombin and fibrinogen. When the calcium of the plasma is precipitated as the oxalate, clotting is not so likely to occur under the conditions of the tests. Blood may be drawn and placed in tubes which have been prepared in the following manner: To 100 ml. of distilled water are added 6 gm. ammonium oxalate and 4 gm. potassium oxalate. One drop (0.05 ml.) of the resulting solution is added to the tube for each 2 ml. of blood to be collected. The solution then may be dried in the tube by placing in an incubator or a desiccator.

EQUIPMENT FOR SEROLOGIC PROCEDURES

This section contains suggestions for laboratory equipment which has been found useful by the authors in performing blood grouping, crossmatching, and related procedures. The discussions are not necessarily comprehensive nor should they be interpreted as excluding other equipment.

Microscope. A compound microscope with low power and high dry objectives is satisfactory. The instrument should have a substage iris diaphragm, an Abbé condenser, and a mirror.

Magnifying Mirrors. Several concave mirrors of the type used under the stage of a microscope will be found useful in observing cell suspensions in test tubes.

Illumination for Serologic Tests. A lamp covered with a plate of ground glass, set in the laboratory table, is convenient for observing agglutination macroscopically in test tubes or on slides. A titration lamp having a fluorescent tube, a shield, and a white background has been found suitable for reading agglutination reactions. It also serves as a source of light for a microscope.

Centrifuge. A table model of microcentrifuge is available at low cost which has proved ideal for serologic tests and the separation of cells from serum in small test tubes. It holds four cups, utilizing the angle principle. It is silent in operation, with little inertia to starting and stopping. A one-speed brushless motor, which requires no supplemental lubrication, furnishes the power. It develops a speed of 1700 revolutions per minute. Only rough balancing of the tubes is necessary. The cells can be separated from the plasma in a tube in one to two minutes.

A centrifuge of high speed is unnecessary for the purpose. The inertia is great and the resulting time taken in acceleration and deceleration is long. Furthermore the initial cost is high and the time and expense of servicing is needlessly expensive.

Water Bath. A water bath operated by electricity is preferable. It should be sufficiently large to accommodate several racks of test tubes. Plastic tented hoods make fairly satisfactory covering to prevent evaporation. Like the metal hoods, they permit some water of condensation to drip into the open tubes. The tubes should be closed with corks.

Dry Heat Oven. Glassware may be dried and sterilized in a dry heat oven. Such equipment is manufactured especially for laboratory use, but an enameled gas oven which is made for domestic use gives excellent service. It may be equipped with a gas regulator. The finish and insulation is superior to many products made for the laboratory.

Refrigeration. If a blood bank is in operation, a compartment of the refrigerator may be employed for the storage of sera and blood specimens. A freezing space is desirable for the storage of grouping and typing sera in the frozen state.

Test Tubes. All or most of the tests for blood grouping, typing, and crossmatching can be performed in test tubes. Serologic test tubes are preferable in size from 7 to 13 mm., inside diameter, and 70 to 100 mm. long. They may be made of borate or soft glass. The borate (Pyrex type) glass is not easily etched by washing and handling, but the amount of breakage depends more upon the thickness of the walls of the tubes than upon the type of glass.

Test Tube Racks. Several test tube racks are desirable for serologic procedures. These should be of sheets of copper, stainless steel, or other metal, and designed to permit shaking of the tubes in the rack. There should be two parallel rows of at least ten holes each.

Glass Slides. A supply of the conventional glass slides for microscopic examinations is necessary. In performing several blood groupings simultaneously, pieces of plate glass are recommended.

Plates 6 by 10 inches (15 by 25 cm.) have been found satisfactory. Lines may be etched on the bottom or cut so that the plate is divided into two columns of ten rectangles, each 1 by 3 inches (2.5 by 7.5 cm.).

Pipettes. For the transfer of serum and cell suspensions by the drop, simple ungraduated pipettes are recommended. They should have the general shape of medicine droppers, but those dispensed by the pharmacist are too thin-walled to withstand much cleaning. Suitable ones with thicker-walled tips may be made by drawing glass tubing out in a flame. One end only should be constricted; the other should retain the full caliber of the tubing. If no shoulder is placed on the larger end, the pipettes may be stacked in a magazine. Various types of rubber bulbs may be procured which fit over the larger ends of the pipettes. The length of the pipettes should be slightly in excess of that of the test tubes so that the bottom of the latter may be reached.

Serologic pipettes which are calibrated in units of 0.01 ml. may be employed for the titration of serum, but these can be eliminated by employing the technique of counting drops from uncalibrated pipettes.

Materials for Labeling. Wax pencils for writing on glass and gummed labels are necessities.

Receptacles for Washing Glassware. These may be made of glass, metal, or earthenware and can be procured in a variety of sizes and shapes.

Blocks for Grouping and Crossmatching. An aid to accuracy and convenience is to have several wooden blocks constructed for crossmatching and blood grouping. Holes are bored and labeled for test tubes and receptacles for reagents.

Magazines for Slides and Pipettes. Wooden or metal boxes may be constructed in which clean slides and pipettes can be dispensed.

Interval and Second Timers. It is convenient to have an interval timer which sounds an alarm after a set interval has elapsed. This clock should record intervals up to at least sixty minutes. A stop watch or other device which accurately registers elapsed seconds is desirable for determining avidity times.

CLEANSING OF LABORATORY GLASSWARE

Meticulous cleanliness of laboratory glassware is essential in serologic work. The principal problem is to remove coagulated protein from the slides, tubes, and pipettes. In addition to freedom from visible dirt, a good criterion of chemical cleanliness is to

observe the way in which clean water drains from the glass. If the water is seen to wet completely the surface of the glass and to drain off in an even film, it may be concluded that the piece is clean.

Immersion in Water. The task of cleaning glassware is lightened materially if protein material is not permitted to dry upon it. Receptacles containing clean tap water or cleaning solution should be placed within reach of the technician's work table and glassware should be immersed as soon as it has been used. It may then soak until opportunity for cleaning is presented. The precaution should be taken to insure the complete filling ot tubes and pipettes which are immersed.

Phosphate Cleaning Solution. A mixture devised by Mr. Charles T. Smith of the State Hygienic Laboratory of Iowa has proved very satisfactory for the removal of blood and wax pencil marks from tubes and other laboratory glassware. A solution is made by adding approximately 100 ml. of powdered trisodium phosphate and 30 ml. of powdered tetrasodium pyrophosphate to the gallon (3.7 liters) of water. The glass may soak overnight in cold solution or it is readily cleaned in an hour or so if the water is heated. The hands should be protected by the wearing of rubber gloves. The glass is then rinsed in water to which has been added a few drops of hydrochloric acid. It is then washed in hot tap water and finally in distilled water. It is essential that all the alkali is removed by neutralization with acid and thorough washing. The glass may then be dried in an oven.

Detergent Solution. Glass receptacles containing blood are first soaked in cold water and the major portion of the blood removed mechanically. Approximately 100 ml. of powdered Calgolac, or similar detergent, is added to the gallon (3.7 liters) of hot water. The glass is permitted to stay in the solution for a few minutes and is then drained, thoroughly rinsed in hot tap water, and finally rinsed in distilled water. This is not an alkaline solution and requires no neutralization.

Sulfuric and Chromic Acid Mixture. This is a dangerously corrosive solution and should not be trusted to unskilled hands. It is not recommended for routine use because of the hazards involved. It should be reserved for the occasional cleaning of pipettes and other glassware which will not yield to the usual methods of cleaning. Several precautions should be observed: (1) Those handling the solution should wear rubber or chemical-resistant aprons, rubber gloves, and goggles. Avoid all contact with skin or clothing. (2) The mixture should be kept in a heavy glass receptacle in a sink. Preferably further protection should be insured by placing it inside a heavy earthenware crock so that

if the glass container should break the acid would not do any damage. (3) In mixing, the sulfuric acid should *always* be poured into the aqueous solution of sodium dichromate and *never* the water into the acid.

Make up a saturated solution of technical grade sodium dichromate. To 35 ml. of the dichromate solution *add cautiously* about 1 liter of commercial concentrated sulfuric acid. This solution may be kept and used many times.

Glassware is immersed momentarily or overnight, as required, then rinsed thoroughly in tap water and distilled water.

BLOOD GROUPING (A-B-O SYSTEM)

Testing blood to determine to which of the four groups, A, B, AB, or O, it belongs is termed *blood grouping*. Usually this is ascertained by testing whether the red cells are agglutinated by either or both of the two grouping sera anti-A and anti-B. Corpuscles which are clumped by the anti-A agglutinin are thus demonstrated to contain the A agglutinogen, whereas those which are agglutinated by the anti-B serum contain the B factor.

Blood grouping is performed on the blood of the prospective recipient and donor before transfusion to establish the *likelihood* of compatibility. Blood grouping is employed to classify the individuals of an organization of potential blood donors in order to save time when the occasion for giving a blood transfusion arises. The determination of the blood group of the child, the mother, and the putative father frequently results in evidence which excludes the man falsely accused of fatherhood. It is often possible to establish by blood grouping the fact that two specimens of blood came from different individuals. Inheritance may be studied by determining the blood groups of members of a family. It is possible to prove fraternal twins if the blood groups are different. When the identity of two babies is confused, as in a hospital, determining the blood groups of the babies and the parents sometimes leads to positive identification.

Anti-A and Anti-B Grouping Sera

Anti-A and anti-B agglutinin sera are the reagents usually employed in the identification of the blood groups. The accuracy of the determinations depends on the quality of the antisera with which the tests are performed. Anyone who has the responsibility for blood grouping should evaluate carefully the sera upon which so much depends.

SOURCES AND PREPARATION OF GROUPING SERA

The anti-A and anti-B agglutinins may be obtained in the sera of human beings, in which they occur naturally, or they may be derived from animals in which they have been generated by immunization.

Unaltered Normal Human Sera. Anti-A serum is obtained from a person belonging to group B. Anti-B serum is derived from one who belongs to group A. Serum containing a mixture of anti-A and anti-B agglutinins is present in group O blood. The sera of prospective donors of suitable group are titrated until a person is found whose agglutinin concentration is relatively high. Blood is collected under aseptic conditions in a sterile vessel, and permit ed to clot during storage at 2° to 5° C. for several hours. Then the clot is separated from the sides of the vessel by shaking it loose or inserting a sterile glass or metal rod which is maneuvered to encircle the clot. The blood is refrigerated for an additional eighteen to twenty-four hours to permit the clot to retract and separate from the serum. When separation is complete, the serum is pipetted off with care to prevent admixture with red cells. The clot and the remaining serum is centrifuged to obtain an additional yield of cell-free liquid. The serum is heated in a water bath for thirty minutes at 56° C. to inactivate complement. Complement is destroyed to prevent the potentiation of any hemolysins which may be present. The clear serum is apportioned to small sterile containers (ampules or bottles) and stored. Some prefer to color the serum as an aid to identification. It is conventional to tint the anti-B serum yellow with 0.015 ml. of 1 per cent solution of acriflavine for every milliliter of serum. The anti-A serum may be colored green by the addition of 0.01 ml. of 1 per cent solution of brilliant green per milliliter of serum.

The preparation of serum from normal human blood possesses three disadvantages. Only the rare donor whose serum is exceptionally potent can be employed. Although anti-A serum naturally may contain a high concentration of anti-A_1 agglutinins, the anti-A agglutinins frequently clump A_2 cells in an unsatisfactory low titer. In most cases the natural titer of agglutinins is far exceeded by that obtained by other methods and from other sources.

Fractionated Normal Human Sera. The isohemagglutinins are associated with the globulins of the plasma. Preparations in which the globulins have been isolated from the plasma therefore have a higher titer per unit volume than native serum or plasma. Thalhimer and Myron[2] produced some increase in the potency of agglutinins per unit volume of fluid by separating the plasma globulin by precipitation with ammonium sulfate. Fractional

if the glass container should break the acid would not do any damage. (3) *In mixing, the sulfuric acid should always be poured* into the aqueous solution of sodium dichromate and *never* the water into the acid.

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Immune Animal Sera. Grouping sera anti-A and anti-B are also prepared by the repeated injection of rabbits with suspensions of human erythrocytes of appropriate blood group. Originally this serum contains not only the desired group-specific agglutinins but also agglutinins which act against all human red cells. These species-specific antibodies must be absorbed before the sera are suitable as reagents. Serum prepared by the injection of group A cells should be absorbed by washed erythrocytes of group B, whereas the anti-B serum is absorbed by group A cells. Rabbit antisera are available commercially in the dry state. The powder is mixed with dry sucrose as a diluent. Care must be taken to exclude moisture from the powdered preparation in order to avoid caking.

Methods of Storage of Grouping Sera

It is generally agreed that blood grouping sera lose potency more slowly in the dry or frozen state than when kept liquid. The method of storage is therefore an important factor in maintaining titer.

Liquid Sera. The agglutinins in sera kept in the liquid state will remain potent for some months if the sterile preparations are refrigerated at 2° to 5° C. The titer diminishes much more rapidly when storage is at room temperature.

Frozen Sera. In the frozen state sera are believed to retain the potency of the agglutinins almost indefinitely. The temperature should be maintained at −15° C. or lower. The reagents may be kept in the freezing compartment of a domestic gas or electric refrigerator if facilities for lower temperatures are not available.

Dried Sera. After drying from the frozen state, sera may be kept at room temperature for one to two years although some deterioration in the potency has been noted at the end of that time. Rabbit antisera and human globulin preparations are available commercially in this form. The moisture content should be kept at less than 1 per cent by employing tight closures on receptacles. The powdered material is dissolved in distilled water or saline solution just before use.

Titration of Anti-A and Anti-B Grouping Sera

Equipment and Materials. Serum to be tested (inactivated at 56° C. for thirty minutes). Reference serum of known acceptable potency (proven satisfactory by previous experience or obtained from the National Institute of Health). Test erythrocytes

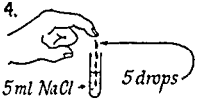
precipitation of the globulins containing isoantibodies was developed by Pillemer, Oncley, Melin, Elliott, and Hutchinson.[3] This results in a sixteenfold concentration of antibodies. Dried globulin made by this method is commercially available. This procedure is adaptable to mass production of agglutinating sera. There is some difficulty in securing acceptably high titers in all lots made from unselected donors.

Immune Human Sera. To increase the natural titer of anti-A or anti-B agglutinins in human beings Witebsky, Klendshoj, and McNeil[4] injected donors of a suitable blood group intravenously with small amounts of protein-free extract of A and B substances The A substance was derived from the stomach of the hog, whereas a mixture of A and B substances was extracted from horse stomach. Four doses of the extract, 0.1, 1.0, 3.0, and 10 ml., were given on succeeding days to donors of the appropriate blood group. Blood was collected from the immunized donor from fifteen to seventeen days after the injections if the titer had sufficiently increased. An increase in titer from 100 to 250 fold was frequently obtained by this procedure. Some persons do not respond to this type of stimulation, however. The present authors have tried this method and are able to confirm the reports. The A and B substances are now obtainable commercially.

A somewhat different method of stimulating agglutinins was introduced by Wiener, Soble, and Polivka.[5] Saliva is collected from a person of suitable blood group who is a secretor. The saliva from a group B person is selected to stimulate a group A donor; a group A person gives saliva for a group B donor. About 20 ml. of saliva is autoclaved at 15 pounds (6.8 kg.) pressure for fifteen minutes to precipitate and coagulate the mucin and protein. The precipitate is removed by centrifugation or filtration, leaving an opalescent fluid which is mixed with four parts of isotonic saline solution. This mixture is apportioned into small bottles and again autoclaved for sterilization. The donor is then injected intramuscularly with about 1 ml. of the solution. Blood is collected and the serum tested about twelve to fourteen days after injection if the titer is sufficiently high. We have obtained an eight to thirty fold increase in potency by this procedure. Occasionally an individual fails to respond to this type of stimulation.

The immune human sera possess several advantages over normal sera for blood grouping. Higher titers are obtained than are likely to be encountered by chance. In particular, the anti-A serum produced by immunization has a greater potency against A_2 cells. The antigen for immunization is readily prepared in any laboratory.

4.

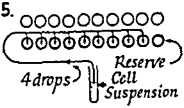


4. *Cell Suspension.* Add 5 full drops (0.25 ml.) of whole blood to 5 ml. of saline in a tube. Use group B blood for testing anti-B serum and either group A_2 or A_2B blood for anti-A serum. The concentration of the cell suspension will be approximately 2 per cent.

5. *Addition of Cell Suspension.* Add 4 drops (0.2 ml.) of the cell suspension to tubes *1* to *9* inclusive in both series. Both tubes *10* are purposely omitted to be kept *in reserve* until the tests are read.

6. *Centrifugation.* Centrifuge the tubes containing the serum-cell mixture for one minute at 500 to 2000 revolutions per minute.

5.



7. *Observation and Recording.* Shake each tube gently and observe with the naked eye whether the cell mass breaks up completely. Record evidence of agglutination as follows:

++++ = cell mass *remains intact* with gentle shaking
+++ = cell mass breaks into *several pieces*
++ = cell mass breaks into *many small pieces*
+ = suspension appears *finely granular*
− = suspension is homogeneous

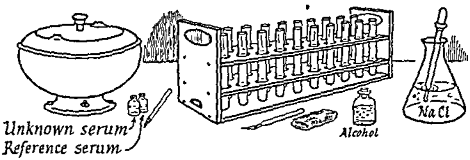
6.



8. *Extension of Dilution Series.* Should all nine tubes be found to contain agglutination, the dilution series may be extended from tube *10* which was previously held in reserve. Label additional tubes with appropriate numbers. To each tube add 4 drops (0.2 ml.) of isotonic saline solution. From tube *10* transfer 4 drops of serum saline solution to tube *11* and repeat throughout the supplemental series, washing the pipette in saline solution between transfers. Then add 4 drops (0.2 ml.) of cell suspension to each tube of serum-saline, centrifuge, and read as before.

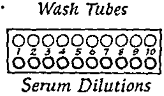
Interpretation. The greatest serum dilution in which macroscopic agglutination is observed is the end point. The titer is the reciprocal of the end point dilution. For example, if the end point

of suitable specificity. Serologic test tubes. Two test tube racks. One drop pipette with rubber bulb. One flask of isotonic saline

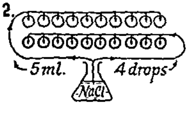


solution (0.9 per cent). Centrifuge. Reading lamp. Wax pencil. Watch or clock showing minutes. Lancet, sponges, and alcohol.

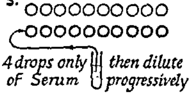
Procedure



1. *Labeling.* Place two parallel rows of ten tubes in each of two racks. Label the tubes of the front row with the numbers *1* to *10* inclusive. These will contain dilutions of the *serum to be tested.* Label the tubes in the other front row with similar numbers, but encircled, to indicate that they will contain dilutions of the *reference serum.*



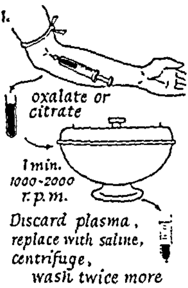
2. *Addition of Saline.* To each tube in both back rows add about 5 ml. or saline solution. In each tube in the front rows place 4 drops (0.2 ml.) of saline.



3. *Serum Dilutions.* With the same pipette. add 4 drops (0.2 ml.) of serum to tube *1*, discard the remainder and wash the pipette three times in the saline of the back tube. After mixing, transfer 4 drops of the serum-saline mixture from tube *1* to tube *2*, and similarly from *2* to *3* and throughout the series, washing the pipette with the saline in the back tube with each transfer. Tube *10* will then contain twice the volume of the others. Set up a similar series of dilutions for the reference serum. The serum dilutions will be $1/2$, $1/4$, $1/8$, $1/16$, $1/32$, $1/64$, $1/128$, $1/256$, $1/512$, and $1/1024$.

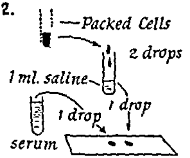
Procedure.

1.



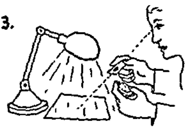
1. *Cell Suspension.* Select a suitable donor as a source of test erythrocytes, group B for anti-B serum, group A_2 or A_2B for anti-A serum. Collect 1 to 2 ml. of blood into a test tube containing oxalate mixture (p. 128). Wash the red cells three times by adding isotonic saline solution to the tube, centrifuging, and discarding the supernatant fluid. To 1 ml. of isotonic saline in a test tube add 2 drops (0.1 ml.) of packed washed red cells, making an approximately 10 per cent cell suspension.

2.



2. *Serum-Cell Mixture.* Place a drop of the serum to be tested near the middle of a slide. About 3 mm. away place a drop of the cell suspension of equal size. Mix the two drops with the bottom of a clean test tube so that a circular area about 25 mm. in diameter is produced.

3.



3. *Agitation.* Start the watch and hold the slide over a source of illumination, tilting the slide back and forth continuously. Observe constantly for signs of agglutination and note the elapsed time when the first sign of macroscopic agglutination occurs. Make five such tests with the unknown serum and five with the reference serum, using the same cell suspension throughout.

Interpretation. Agglutination is adjudged to occur when distinct clumps are visible to the naked eye. At least some of the cell masses should be 1 mm. in diameter at the end of 120 seconds. The average of five tests is considered the avidity time and this is compared with the average for the five tests on the reference serum. The avidity of the unknown serum is considered satisfactory if the avidity time is less or equal to that of the reference serum (p. 127). Lacking a reference serum which is authentic, the following avidity times are acceptable to the National Institute of Health, provided the tests be performed precisely as described:

occurs in a dilution of 1/256, the titer is 256. No correction in the dilution should be made for the added volume of cell suspension (p. 126).

The titer of the unknown serum should be compared with that of the reference serum which is used in the parallel test. A difference in titer of one dilution is not significant. The potency of the unknown serum can be expressed in terms of the reference serum as *R*, *2R*, and *0.5R* (p. 127). Lacking an authoritative reference serum, the following titers are considered acceptable by the National Institute of Health, if determined precisely by the technique described here:

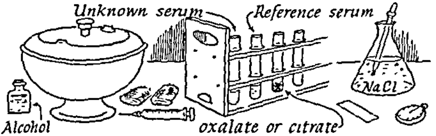
	Cells	*Titer*
Anti-A serum	A_1	256
" "	A_2	128
" "	A_2B	64
Anti-B "	B	256

Sources of Error. (1) The use of test erythrocytes of the wrong blood group or subgroup. (2) The omission or doubling of the transfer of serum in making the dilution series. (3) Hemolysis from chemicals in the saline solution or on the glassware. (4) Mislabeling.

Checking. The group or subgroup of the test erythrocytes should be carefully confirmed. Hemolysis usually can be suspected or excluded by observing the results of tests of the reference serum in the same solutions and glassware. The entire titration should be repeated.

Avidity of Anti-A and Anti-B Grouping Sera

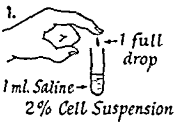
Equipment and Materials. Serum to be tested (inactivated at 56° C. for thirty minutes). Reference serum of known acceptable potency (proven satisfactory by previous experience or obtained



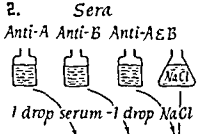
from the National Institute of Health). Test erythrocytes of suitable specificity. Several microscope slides or small glass plates. Reading lamp. One drop pipette for each serum and cell suspension. A stop watch. Wax pencil. Isotonic saline solution. Sterile syringe, needle, tourniquet, sponges, and alcohol.

blood containing both anti-A and anti-B agglutinins. Serologic test tubes. Isotonic saline solution (0.9 per cent). Drop pipettes. Centrifuge. Microscope. Wax pencil. Lancet, sponges, and alcohol.

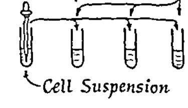
Procedure



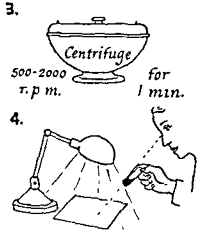
1. *Cell Suspension.* Make a 2 per cent cell suspension of the erythrocytes to be tested by adding 1 full drop of blood to 1 ml. of isotonic saline solution in a test tube. Cells loosened from a clot may be employed in saline, the suspension being prepared with the same turbidity as by the other method.



2. *Serum-Cell Mixture.* Label a serologic test tube *A*, another *B*, and a third *A & B* (optional). Place 1 drop of cell suspension in each of the two or three tubes. Add 1 drop of saline solution to each tube. To tube *A* add 1 drop of anti-A serum, to tube *B* one drop of anti-B serum, and to tube *A & B* 1 drop of anti-A & B serum.



3. *Centrifugation.* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.



4. *Observation.* Immediately after centrifugation tilt the tubes gently and observe whether the cell mass breaks up completely. Readings may be checked by microscopic examination.

Interpretation. The blood group to which the cells belong is determined by the presence or absence of agglutination with the known antisera:

Blood Group of Cells	Anti-A	*Sera* Anti-B	Anti-A & B
A	+	−	+
B	−	+	+
AB	+	+	+
O	−	−	−

	Cells	Maximum Time Before Agglutination is Visible
Anti-A serum	A_1	15 seconds
" "	A_2	30 "
" "	A_1B	30 "
" "	A_2B	45 "
Anti-B "	B	15 "

Sources of Error. (1) The use of test erythrocytes of the wrong blood group or subgroup. (2) The use of test erythrocytes in suspensions with a concentration other than 10 per cent. Thinner suspensions prolong the time of agglutination.

Checking. The specificity of the test erythrocytes should be checked. The cell suspensions should be made again with special attention to concentration and the tests repeated.

Specificity of Anti-A and Anti-B Grouping Sera

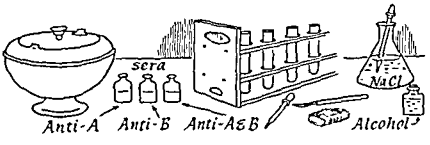
Grouping serum should be tested for specificity before it is put to routine use. Anti-A serum should be tested against several bloods belonging to subgroup A_2 and A_2B to insure the identification of these weakly reacting cells. Sera should have a low titer of cold agglutinins. There should be no tendency of the agglutinating sera to form rouleaux because such a property seriously interferes with the interpretation of results. Isohemolysins should be inactivated by the destruction of complement by heating the serum at 56° C. for thirty minutes.

Determination of Blood Groups (A-B-O System)

Centrifuge Method for Blood Grouping

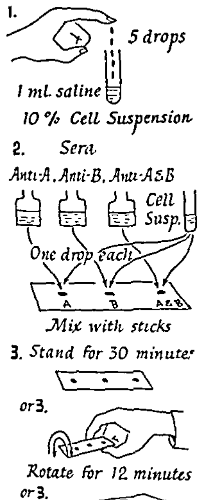
This method is considered by many workers as the most satisfactory and definitive. It gives sharp readings even when the grouping sera are not very potent. Thinner cell suspensions may be employed. Several tests may be performed more or less simultaneously because they are carried out in test tubes.

Equipment and Materials. Anti-A and anti-B grouping sera evaluated as on pages 135 and 138. Optional: serum from group O



pencil. Microscope slides, either plane or well type.* Shaking machine (optional). Lancet, sponges, and alcohol.

Procedure



1. *Cell Suspension.* Make a 10 per cent suspension of the erythrocytes of the unknown blood by adding 5 drops of whole blood to 1 ml. of isotonic saline solution, or make a similarly turbid suspension from red cells loosened from a clot.

2. *Serum-Cell Mixture.* Mark an *A* on the left position of a slide and a *B* on the right position. Place 1 drop of anti-A serum in the *A* position and 1 drop of anti-B serum on the *B* position. Add 1 drop of cell suspension to each drop of serum and mix with wooden applicators or toothpicks.

3. *Time of Cell Contact.* Alternatives: *No Agitation.* Permit the slide to stand for thirty minutes at room temperature. Then tilt back and forth five or six times until the suspension is thoroughly displaced. Observe the presence or absence of agglutination. Check with the microscope. *Manual Agitation.* Tilt the slide back and forth for from three to twelve minutes, watching for signs of agglutination. Check observations with the microscope. *Mechanical Agitation.* Place well or paraffin ring slides on a Boerner shaker and agitate for from three to twelve minutes.

4. *Observation.* Observe for agglutination with the naked eye and the microscope.

* Paraffin ring slides may be prepared as follows: Make a double loop of No. 28 gauge iron wire by winding two turns around a test tube (diameter 12 to 15 mm.). Twist the ends together and bend the resulting stem at right angles to the plane of the circle. Bind the two loops together by winding with linen thread. Dip the circle in melted paraffin and then touch it lightly to a clean microscope slide so that the circle of paraffin is deposited on the slide.

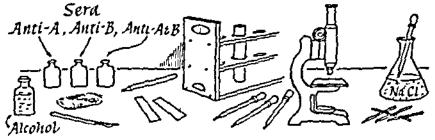
Sources of Error. It must be realized that the absence of agglutination has as much weight in the interpretation as its presence. *Causes of false negative reactions* are (1) mislabeling, (2) weak grouping sera, (3) destruction of agglutinates by hemolysins when complement has not been inactivated in the sera, (4) hemolysis from chemicals in solutions or on glassware, (5) excessively heavy cell suspensions which absorb all agglutinins, thus preventing agglutination, and (6) slight sensitivity of agglutinogens encountered in the erythrocytes of the newborn and in the subgroups A_2, A_2B, A_3, and A_3B. *Causes of false positive reactions* are (1) mislabeling, (2) rouleau formation, (3) cold hemagglutinins in high titer, (4) anti-Rh or anti-Hr agglutinins in high titer, (5) bacteriogenic agglutination, and (6) clotting.

Checking. There is no complete check in the method itself. A partial check is obtained by employing the grouping serum anti-A & B. If clumping is caused by anti-A & B serum but by neither of the others, the discrepancy should be solved. This will not detect all the errors. Other discrepancies may be demonstrated by the following: (1) Repetition of the tests with the same grouping sera will show errors in mislabeling or incidental technique. (2) Performance of the tests employing grouping sera from other sources will demonstrate weak grouping sera. (3) Testing the serum of the unknown blood against known group A and group B cells will give further proof of the blood group.

Slide Method for Blood Grouping

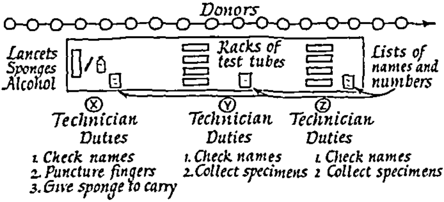
This procedure is considered more desirable by some when only occasional tests are performed. The advantages are that it requires a minimum of laboratory equipment and the maneuvers are exceedingly simple. But the method lacks the quick maximum contact of erythrocytes which is obtained by centrifugation, so that frequently there is some doubt as to how much time to allow for agglutination to occur (p. 121).

Equipment and Materials. Anti-A and anti-B grouping sera evaluated as on pages 135 and 138. Isotonic saline solution (0.9 per cent NaCl). Drop pipettes. Test tube. Microscope. Wax



The procedure to be described depends on two principles: (1) The potency and specificity of the grouping sera must be established beyond doubt. (2) Each blood grouping must be performed twice, as independently as possible, and the results of the two tests checked against each other.

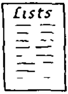
Equipment and Materials. Serologic test tubes, two for each person to be tested. Test tube racks. Isotonic saline solution (0.9 per cent NaCl), 1 ml. for each test tube, plus an additional 1000



ml. Several drop pipettes. Six or more glass plates as described on page 129. Wax pencils. Microscope. Supplies of alcohol, sponges, and lancets. Long table. Technicians X, Y, and Z.

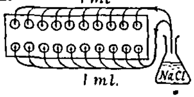
Procedure

1



1. *Name List.* Prepare a clearly written list in triplicate of the names of the persons whose blood is to be tested. Assign to each name a laboratory number, and record the numbers opposite the names on the list.

2.



2. *Labeling Tubes.* Prepare duplicate sets of test tubes in separate racks, marking the laboratory numbers on the tubes. A complete series of numbered tubes should be in each set, in order. In each tube place about 1 ml. of isotonic saline solution.

Interpretation. The presence or absence of agglutination with the known antisera is interpreted as on page 141.

Sources of Error. In addition to those sources of error listed on page 142, there are others inherent in this method. False negative reactions may be due to the fact that not sufficient time has been allowed for the sensitized cells to make mutual contact. The appearance of dried or sedimented cell suspensions on the slide may be erroneously interpreted as agglutination.

Checking. The same principles apply as on page 142. In addition, false negative reactions may be converted to agglutination by allowing the cell-serum mixtures longer time in which to act or by submitting them to more vigorous agitation.

Mass Blood Grouping

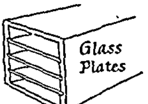
Occasionally it is desired to perform blood grouping on large numbers of persons more or less simultaneously. Although the methods described on page 140 are efficient for testing ten or less specimens, they become cumbersome for larger numbers. There is another difference. In the blood transfusion laboratory blood grouping of the donor and recipient frequently is performed as an immediate preliminary to transfusion so that the results of the tests are desired promptly. In the case of mass blood grouping there is no immediate demand for the results. For this reason different methods are feasible.

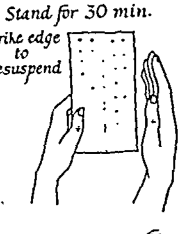
A study was made[6] of the numbers and types of errors scored by various workers when blood grouping was performed on from thirty to 432 specimens in a series. Potent grouping sera were employed in all tests. Under these conditions the most common errors were in mislabeling and otherwise confusing specimens. No person made less than 1 per cent errors and occasionally the incidence of mistakes with some technicians was as high as 10 per cent.

Also apparent was the fact that the method of grouping which produced the quickest result for the single specimen, as in testing prior to transfusion, was not necessarily the most time-saving when large numbers of specimens were presented. In this case the centrifuge method was found to be cumbersome and time-consuming.

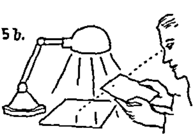
Time was easily saved by setting up the tests on slides and permitting them to stand while other tests were being performed or read.

5a.





5b.



grouping sera in the rectangle numbered *1*. Empty the pipette, wash three times with saline from the beaker, expelling it into the initially empty beaker. Use the same pipette for transferring the other cell suspensions similarly.

5. *Reading.* Permit the plate to stand motionless at room temperature for thirty minutes, during which time several other plates may be set up or read. After the plate has stood for thirty minutes, take it up and hold it over a white surface which is illuminated obliquely. Holding the plate with the left hand, strike the edge with the right hand several times to break up the sedimented cell masses, and observe for agglutination. If doubt exists, place the plate under the microscope and inspect.

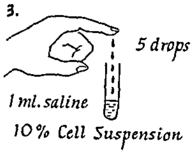
6. *Comparison.* Compare the records of the results of the two series of tests. Check the specimens which are the subjects of discrepancies.

Interpretation. The presence or absence of agglutination with the known antisera has the significance stated on page 141.

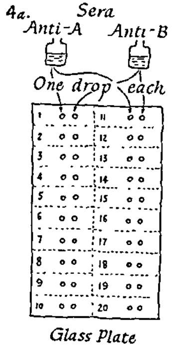
Sources of Error. The principal errors in this procedure are in mislabeling or otherwise confusing blood specimens. Occasionally, when the humidity is low, there is difficulty because the serum-cell mixtures dry during the time allowed for sedimentation.

Checking. If the specificity and potency of the antisera are assured, the best check is the comparison of the results of tests on two separate cell suspensions, taken independently, and tested separately.

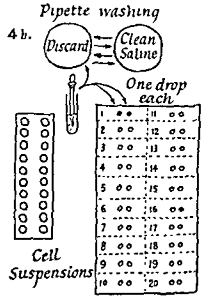
If a minimum of 1 per cent error is allowed to each worker, two technicians should make ten errors each in the same 1000 specimens. The probabilities that both will err on the same specimen are $\frac{10}{1000} \times \frac{10}{1000} = \frac{1}{10{,}000}$. Since each test consists of the reactions of two sera, read separately, the chances that the two workers will make the same error on the same specimen are approximately $\frac{1}{10{,}000} \times \frac{1}{2} = \frac{1}{20{,}000}$ or one in 20,000. Some workers have advocated testing the sera of the unknown bloods routinely against



3. *Collection of Specimens.* Behind a long table seat three technicians. Place a copy of the name list on the table before each technician. Before Technician X place a supply of alcohol, sponges, and lancets. Before Technician Y place a complete set of numbered test tubes containing saline. Place the duplicate set of tubes before Technician Z. Let Technician X call the names on the list and, as the persons present themselves, he punctures the finger of each and gives him a sponge to carry. The donor passes on to Technician Y who checks the name and number, selects the properly numbered tube, and expresses from 3 to 5 drops of blood into the tube. Have the donor then pass on to Technician Z where the same procedure is repeated, checking the name again.



4. *Serum-cell Mixtures.* Each of the duplicate series of specimens should be tested separately, either by two technicians working independently or by the same worker testing the two series in succession. The same grouping sera may be employed for the two series or different sera may be employed for each series.



Take a clean glass plate, marked off in 20 rectangles, 1 by 3 inches (2.5 by 7.5 cm.). Mark the serial number of the blood specimens to be tested in the upper left corner of each rectangle. Place two beakers (250 to 500 ml. capacity) back of the glass plate, the right filled with clean saline solution and the left empty. Place 1 drop of anti-A grouping serum near the left end of each of the twenty rectangles. Similarly, place 1 drop of anti-B serum near the right end of each rectangle. Pick up tube *1* with the left hand, draw up some of the cell suspension in a drop pipette held in the right, and place 1 drop on each of the

basis of chance, one fifth of the A bloods tested should belong to this subgroup.

About 5 ml. of blood is collected from a donor of subgroup A_2 and is citrated or oxalated. The cells are washed three times with sterile isotonic saline solution. About 1 ml. of washed packed A_2 erythrocytes is added to 3 ml. of sterile anti-A grouping serum. The mixture is permitted to stand at room temperature for thirty to sixty minutes. The cells are then separated from the serum by centrifugation and the serum is tested for anti-A agglutinins. The test is made by adding 1 drop of the absorbed serum to a tube containing 1 drop of 2 per cent suspension of A_1 cells and another containing A_2 cells. One drop of saline is added to each tube and the mixtures are centrifuged for one minute at 1000 to 2000 revolutions per minute. The absorbed serum should agglutinate A_1 cells but not A_2 erythrocytes. If the serum still agglutinates A_2 cells the absorption is repeated with a smaller volume of cells than before. The absorbed serum is stored in sterile containers at low temperature. The discussion on storage and potency on pages 125, 126, and 135 also apply to absorbed serum.

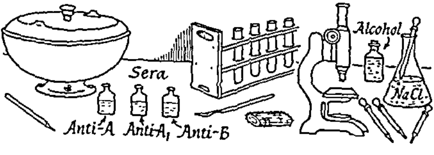
Determination of Subgroups of A and B

When erythrocytes have been demonstrated to belong to groups A or AB by their reactions to anti-A and anti-B grouping sera, the agglutinogen A_1 may be identified by its agglutination by anti-A_1 serum. By inference, A agglutinogens which are not agglutinated are presumed to be A_2.

Centrifuge Method for Grouping A_1 and A_2

As in the determination of the blood groups, this method is preferred by many workers.

Equipment and Materials. Serologic test tubes. Isotonic saline solution (0.9 per cent NaCl). Grouping sera anti-A, anti-A_1, and



anti-B. Drop pipettes. Wax pencil. Centrifuge. Microscope. Lancet, sponges, and alcohol.

cells of group A and B as a check on the results obtained with known antisera. This method has the disadvantage of not being more accurate than two tests of the cells with known antisera and is far more time-consuming. Furthermore, in such tests one must deal with unselected sera, many of which have low potency and therefore cause difficulty in interpretation of the tests. This procedure should be reserved for the occasional blood about which doubt has arisen as a result of the other tests.

BLOOD GROUPING (SUBGROUPS OF A AND AB)

In routine blood grouping for transfusion the subgroups of A and AB are not differentiated and such a classification is unnecessary. Occasionally, however, it is desirable to assign definitely a blood to the subgroup of A to which it belongs. Such a procedure may be necessary when an irregular or unexplained agglutinin is demonstrated in crossmatching prior to transfusion. Since the subgroups of A are inherited factors, their demonstration enhances the possibility of the exclusion of falsely accused men in cases of disputed paternity. Two blood specimens of group A or AB can be demonstrated to have come from different sources if the A agglutinogens are of different subgroups.

Anti-A_1 Grouping Serum

Sources and Preparation of Anti-A_1 Serum

It was pointed out previously (Chap. 4) that the sera from groups B and O contain a mixture of *anti-A agglutinins* which clump both A_1 and A_2 cells and *anti-A_1 agglutinins* which act against only A_1 erythrocytes. If the serum from group B blood be absorbed with A_2 cells, the anti-A agglutinins are removed, leaving only the anti-A_1 antibodies. The resulting reagent is commonly known as *absorbed B serum* or *absorbed anti-A serum.*

The absorbed serum may be purchased from a reliable supplier but it is quite easily prepared in the laboratory. The first step is to select blood cells belonging to the subgroup A_2. If absorbed serum is available, then blood specimens which are determined as belonging to group A by the routine grouping procedures are tested for their agglutinability with the anti-A_1 serum. Those cells which do not react presumably belong to subgroup A_2. If no identifying serum is available, the erythrocytes of twenty or thirty blood specimens which have been shown to belong to group A should be tested with an anti-A serum which is not very potent. The weakly agglutinated cells presumably belong to subgroup A_2. On the

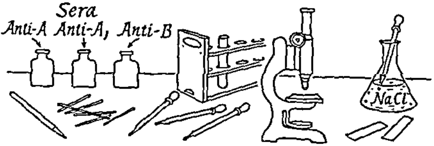
Sources of Error. The absence of agglutination has as much weight in the interpretation as its presence. *Causes of false negative reactions* are (1) weak grouping sera, (2) mislabeling, (3) destruction of the agglutinates by hemolysins in the presence of complement which has not been inactivated, (4) hemolysis from chemicals in the saline solution or on the glassware, (5) excessively heavy cell suspensions which absorb all the agglutinins, thus preventing agglutination, (6) slight sensitivity of the cells of the newborn, and (7) the presence of agglutinogens A_3. *Causes of false positive reactions* are (1) mislabeling, (2) rouleau formation, (3) cold hemagglutination, (4) incompletely absorbed anti-A_1 serum, (5) bacteriogenic agglutination, and (6) clotting.

Checking. There are no complete checks in the method itself. Repetition of the tests with the same grouping sera will detect errors in mislabeling or incidental technique. Retesting the cells with antisera from other sources and the employment of cells of known subgroups as controls should demonstrate unsatisfactory sera.

SLIDE METHOD FOR GROUPING A_1 AND A_2

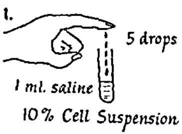
This method is considered more convenient for the occasional test.

Equipment and Materials. Serologic test tube. Isotonic saline solution (0.9 per cent NaCl). Grouping sera anti-A_1, anti-A, and



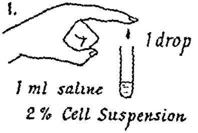
anti-B. Drop pipettes. Wax pencil. Microscope slides. Microscope. Lancet, sponges, and alcohol.

Procedure

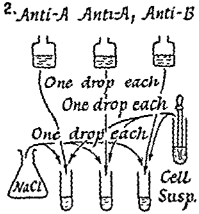


1. *Cell Suspension.* Make a 10 per cent suspension of the unknown erythrocytes by adding 5 drops of blood to 1 ml. of saline solution in a tube.

Procedure



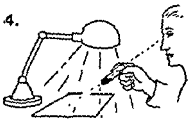
1. *Cell Suspension.* Make a 2 per cent suspension of the erythrocytes to be tested by adding 1 full drop of blood to 1 ml. of isotonic saline solution in a test tube.



2. *Serum-Cell Mixture.* Label a tube A, another A_1, and a third B. Place 1 drop of cell suspension in each tube. Add 1 drop of saline solution to each. To tube A add 1 drop of anti-A serum, and likewise anti-A_1 and anti-B sera to the proper tubes.



3. *Centrifugation.* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.



4. *Observation.* After centrifugation shake the tubes gently and determine whether the cell mass breaks up completely or agglutination is present. Readings may be checked by microscopic examination.

Interpretation. The subgroup to which the cells belong is determined by the presence or absence of agglutination with the various antisera:

Blood Group of Cells	Sera Anti-A	Anti-A_1	Anti-B
A_1	+	+	−
A_2	+	−	−
B	−	−	+
A_1B	+	+	+
A_2B	+	−	+
O	−	−	−

BLOOD TYPING (M-N SYSTEM)

Determination of the types in the M-N system is rarely performed in blood transfusion. Occasionally two bloods of the same A-B-O group may be shown to have come from different individuals by the demonstration of the M-N types. Since the laws of inheritance of the M and N agglutinogens are known, typing is sometimes useful in cases of disputed parentage.

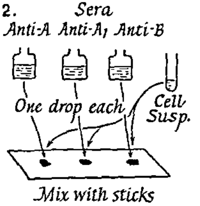
Anti-M and Anti-N Typing Sera

Sources and Preparation of Sera

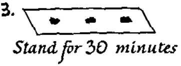
Anti-M and anti-N sera usually are prepared by the immunization of rabbits with human erythrocytes of the proper M-N type belonging to group O. Good sera may be obtained commercially in either the liquid or dry form. The powdered serum is packaged in glass vials, with compartments containing calcium chloride to absorb the moisture. They must be kept tightly stoppered.

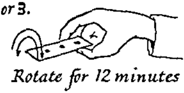
The preparation of anti-M and anti-N serum is difficult and the reader is cautioned that considerable experience is required. The procedure is a long-term project and suitable quarters and facilities should be available to care for many rabbits for a number of months. Wiener[7] advised the following method: To immunize twelve rabbits, collect about 25 ml. of blood from a donor belonging to group OM or ON, as desired. The blood is added in correct proportions to one of the preservative mixtures (Chap. 13). The blood mixture is apportioned equally to seven sterile tubes and stored at 2° to 5° C. until needed. A course of seven daily injections is given to each animal. Each day the red cells from one of the tubes are washed twice in sterile saline solution and injected intravenously, one-twelfth part to each rabbit. After a rest of seven to twelve days, another volume of blood is collected from the same donor, processed as before, and administered in seven daily injections, the first dose intraperitoneally, the remainder intravenously. The animals' sera are tested one week after the last injection Good anti-N sera frequently are prepared after three or four courses of injections, although some animals require more. Anti-M sera are more difficult to produce. It is advisable to reinject, in further courses after a rest of two or three months. A large number of animals is advised because of the mortality from intercurrent infection or anaphylaxis.

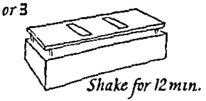
After a satisfactory agglutinin titer has been obtained, the animal is exsanguinated or a volume of blood is withdrawn sufficient for the need. The serum is separated from the cells in the usual manner



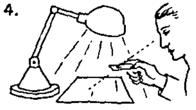
2. *Serum-Cell Mixture.* Mark an *A* on the left position of a slide and an A_1 on the right position. Place 1 drop of anti-A serum in the designated spot and 1 drop of anti-A_1 serum in the right position. Add 1 drop of cell suspension to each drop of serum and thoroughly mix with wooden applicators or toothpicks Make a similar preparation on another slide using anti-B serum.







3. *Time of Cell Contact.* Alternatives: *No Agitation.* Permit the slide to stand for thirty minutes at room temperature. Then tilt back and forth five or six times until the suspension is thoroughly remixed. Observe the presence or absence of agglutination, using the microscope if necessary. *Manual Agitation.* Tilt the slide back and forth continuously for from three to twelve minutes, watching for signs of agglutination. *Mechanical Agitation.* Shake the slides on a Boerner shaker for three to twelve minutes and observe for signs of agglutination.

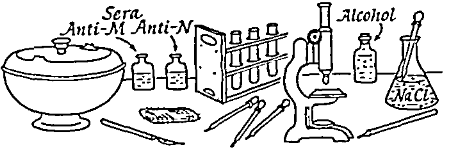


4. *Observation.* Inspect for evidence of agglutination.

Interpretation. The presence or absence of agglutination with the known antisera is interpreted as on page 150.

Sources of Error. In addition to those mentioned on page 151, there is the uncertainty of knowing whether sufficient time has been permitted for the erythrocytes to make mutual contact. Occasionally, when the tests are performed when the humidity of the air is low, drying may cause erroneous conclusions to be drawn.

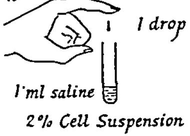
Checking. The same principles apply which are listed on page 151.



(0.9 per cent NaCl). Drop pipettes. Centrifuge. Microscope. Wax pencil. Lancet, sponges, and alcohol.

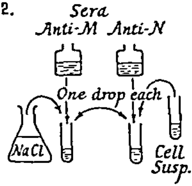
Procedure

1.



1. *Cell Suspension.* Make a 2 per cent suspension of the erythrocytes to be tested by adding 1 full drop of whole blood to 1 ml. of saline solution in a test tube. Make similar suspensions of cells known to belong to types M and N.

2.



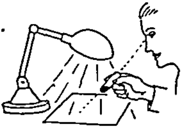
2. *Serum-Cell Mixture.* Label a test tube *M* and another *N*. Place 1 drop of the unknown cell suspension in each tube. To each tube add 1 drop of saline solution. To tube *M* add 1 drop of anti-M serum and to tube *N* add 1 drop of anti-N serum. Make up similar sets of tubes using the M cells and the N cells.

3.



3. *Centrifugation.* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.

4.



4. *Observation.* Shake the tubes gently to determine whether the cell mass breaks up completely. Readings may be checked with the microscope.

(p. 133). In addition to the anti-M or anti-N agglutinins this serum will contain agglutinins which react against all human erythrocytes, the species-specific agglutinins. The latter must be absorbed before the material is suitable as a reagent. Anti-M serum is absorbed by ON erythrocytes and anti-N serum by OM cells. The complement is first inactivated by heating the serum at 56° C. for thirty minutes. The serum is then diluted from fifteen to twenty-five times with sterile isotonic saline solution and mixed with a half volume of washed erythrocytes of the proper group and type. This mixture is allowed to stand for thirty minutes at room temperature. The cells are separated from the diluted serum by centrifugation and the serum is titrated against cells of group OM, ON, and OMN (p. 135). If nonspecific agglutination is demonstrated a second absorption is performed and the serum retested. This is continued until all the nonspecific agglutinins have been removed. It is more difficult to prepare anti-N sera than anti-M because some of the anti-N antibodies are removed during the nonspecific absorption.

METHODS OF STORAGE OF SERA

The sera may be stored in the liquid state at 2° to 5° C. for periods varying from a few months to several years. The loss of potency seems to vary with the animal from which it is derived. The potency is probably retained indefinitely in the dry or frozen state.

POTENCY OF SERA

The sera may be titrated in a manner similar to that used for anti-A and anti-B sera (p. 135). No standards have been accepted as minimal for these sera but it is seldom possible to obtain anti-N sera with a titer higher than 16 although anti-M sera are frequently encountered with titers of 128.

The avidity of the sera may be measured by the methods described on p. 138, but no data are available to state what the minimal acceptable standards should be.

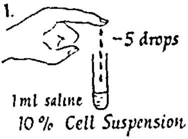
Determination of Types M, N, and MN

CENTRIFUGE METHOD OF TYPING M AND N

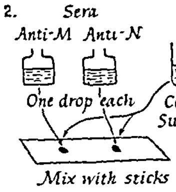
This method is preferred for technical reasons but is not readily adaptable when dried antisera are employed.

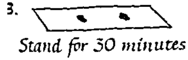
Equipment and Materials. Serologic test tubes. Anti-M and anti-N typing sera. Type M and N cells. Isotonic saline solution

Procedure

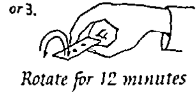


1. *Cell Suspension.* Make a 10 per cent suspension of the erythrocytes to be tested by adding 5 drops of whole blood to 1 ml. of saline solution in a test tube. Make similar suspensions of known M and N cells.

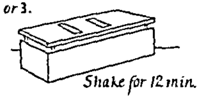


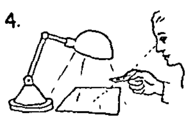


2. *Serum-Cell Mixture.* Label the left position of a slide *M* and the right *N*. At each end place 1 drop of cell suspension. Add 1 drop of anti-M serum to the cells in the labeled position and 1 drop of anti-N serum to the cells so designated. Mix with applicators or toothpicks. If the sera are in the dry state, take the amount of powder which can be held on the tip of a toothpick and mix with the cell suspension until the serum is dissolved Make similar mixtures with the known cells.



3. *Agitation.* Tilt the slide back and forth for several minutes. The exact time should be determined by the potency of the sera. If the known red cells are tested first, the time of reaction will be evident.





4. *Observation* Note the presence or absence of agglutination with the unaided eye and the microscope.

Interpretation. The type to which the cells belong is determined by the presence or absence of agglutination with the two antisera:

Blood Group of Cells	*Sera* Anti-M	Anti-N
M	+	−
N	−	+
MN	+	+

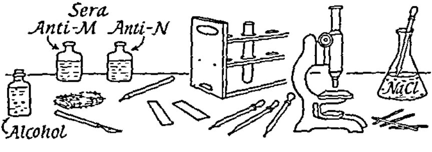
Sources of Error. The absence of agglutination has as much weight in the interpretation as its presence. It should be noted that no human red cells are inagglutinable by both antisera. *Causes of false negative reactions* are (1) mislabeling, (2) weak typing sera, (3) destruction of the agglutinates by hemolysins and complement which has not been inactivated, (4) hemolysis from chemicals in the saline or on the glassware, and (5) excessively heavy cell suspensions which absorb all the agglutinins. Also, (6) MN cells react normally less well than N cells to anti-N serum. Such agglutination may be overlooked and the cells erroneously diagnosed as belonging to type M. (7) N_2 cells may not react with weak anti-N serum. *Causes of false positive reactions* are (1) mislabeling, (2) rouleau formation, (3) unabsorbed species-specific agglutinins in the animal antisera, (4) cold hemagglutination, (5) bacteriogenic agglutination, and (6) clotting.

Checking. Repetition of the tests with the same reagents will reveal errors in mislabeling and incidental technique. Performance of the tests with other antisera will demonstrate faulty reagents.

SLIDE METHOD OF TYPING M AND N

This method is satisfactory when relatively small amounts of dry antisera are to be employed.

Equipment and Material. Anti-M and anti-N typing sera. Microscope slides. Serologic test tubes. Erythrocytes of type M



and N. Drop pipettes. Wooden applicators or toothpicks. Isotonic saline solution (0.9 per cent NaCl). Wax pencil. Microscope. Lancet, sponges, and alcohol.

of agglutinins quickly rose and the donors were bled when a satisfactory potency was attained. It was emphasized that only male donors or females who had passed the menopause should be employed for the purpose. Of course, such persons should understand that there is a hazard if they should later receive transfusions of blood without regard to Rh type.

Immune Animal Sera. Only anti-Rh_0 serum has been produced by the immunization of animals. Originally such serum was made by the injection of cells from the rhesus monkey into rabbits and guinea pigs. These animals have not yielded sera which are reliable for routine use. Recently goat serum has been produced on a commercial scale, but the titer and specificity have not been satisfactory. All animal anti-Rh sera have the disadvantage of *agglutinating the red cells of all newborn infants.*

Methods of Storage of Sera

Anti-Rh sera are more labile than anti-A, anti-B, anti-M, and anti-N sera.

Liquid Sera. The agglutinins in sera kept at room temperature deteriorate rapidly. They retain their potency for some months with storage at 2° to 5° C. Their potency should be checked frequently.

Frozen Sera. Sera kept at −15° C. or lower retain their potency for months or possibly for years.

Dried Sera. Reagents kept in the dry state with less than 1 per cent moisture apparently retain their strength for months or years but there are inexplicable exceptions to this statement. It is considered safer to store the dried sera below 5° C.

Titration of Sera Without Blocking Antibodies

Anti-Rh typing serum without blocking antibodies may be titrated by testing serial dilutions against a 2 per cent suspension of suitable erythrocytes in *saline solution.* The serum is also diluted with saline. The procedure is similar to that described on page 135 with the exception that the serum-cell mixtures are incubated at 37° C. for sixty minutes before centrifugation. The serum containing anti-Rh antibodies should not be inactivated with heat to destroy complement. In inspecting the tubes for the presence of agglutination, they should be removed from the centrifuge, one at a time, and gently tilted and partially rotated in a good light. Attention is directed to the manner in which the cell mass breaks up. The agglutinates formed by the anti-Rh agglutinins are frequently small and fragile, in contrast to those formed by anti-A or anti-B

Interpretation, Sources of Error, and Checking. Consult page 156.

BLOOD TYPING (Rh SYSTEM)

Blood typing with reference to the Rh system is performed either to determine the presence or absence of any Rh agglutinogens in red cells or to classify the cells with respect to the subtypes of Rh. The question of whether the blood is Rh positive or Rh negative occurs more or less routinely before blood transfusion whereas the problem of determining the specific subtypes is raised only when the mechanism of isosensitization is being investigated.

Anti-Rh Typing Sera

SOURCES AND PREPARATION OF SERA

Good anti-Rh typing sera are still difficult to obtain. Most varieties must be derived from the rare human beings who have become sensitized either by transfusion or pregnancy. Only the anti-Rh_0 serum has been produced by the immunization of animals.

Sera From Persons Accidently Sensitized. Anti-Rh_0, anti-Rh_0', anti-Rh_0'', anti-rh', and anti-rh'' sera can be obtained from human beings who have been sensitized to the proper antigen. The anti-Rh_0' agglutinins are the most commonly encountered in accidental sensitization whereas the anti-rh' and anti-rh'' are the most rare. In many cases the titer is so low as to be inadeuqate for use as typing serum. The discovery of the properties of the blocking antibodies has made possible the use of techniques which permit employment of many sera which formerly were not considered adequate as reagents. When a person is found to possess anti-Rh agglutinins of suitable potency blood is collected and processed into serum as described on page 133. Anti-A and anti-B agglutinins may be neutralized by the addition of an extract of A and B group-specific substances. Sera designed to be employed *as anti-Rh reagents should not be inactivated by heat to destroy* the complement.

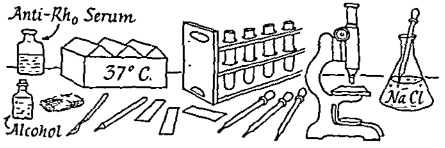
Sera from Persons Purposely Sensitized. Several groups of workers[8,9] have reported the production of satisfactory sera by the repeated injection intravenously of small doses of human red cells of appropriate type into human beings who had previously been sensitized. Three or four injections of Rh-positive blood were given at intervals of three to five days in doses from 1 to 5 ml. The titer

negative. The question arises as to which antiserum to employ for this purpose. A strict interpretation would require that Rh-negative cells be demonstrated to be inagglutinable to all three sera anti-Rh_0, anti-rh′, and anti-rh″. Since the serum anti-Rh_0′ is most commonly encountered in clinical isosensitization and is the most polyvalent, some have employed it in routine typing. Thus all the subtypes of Rh react except the rare rh″ which is classified as Rh negative. Wiener has pointed out, however, that Rh_0 is the most potent antigen in the system and that persons belonging to rh′, rh″, or rh′rh″ may be sensitized to it. He has advised the employment of anti-Rh_0 serum as the routine typing agent, thus classifying rh′, rh″, and rh′rh″ as Rh negative. Recipients who belong to these subtypes will therefore receive Rh-negative blood. In the present state of our knowledge, this seems the safest procedure.

CENTRIFUGE METHOD FOR TYPING RH POSITIVE AND RH NEGATIVE

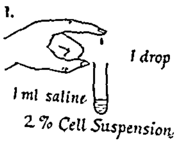
This technique is preferred to the slide method by many workers because it permits manipulation of the serum-cell mixture, washing the cells, and so forth, without loss. It has the disadvantage of being somewhat slower than the slide method.

Equipment and Materials. Serologic test tubes. Isotonic saline solution (0.9 per cent NaCl). Anti-Rh_0 typing serum (without blocking antibodies). Erythrocytes known to belong to rh and Rh_0.



Water bath adjusted to 37° C. Microscope slide. Microscope. Wax pencil. Drop pipettes. Lancet, sponges, and alcohol.

Procedure



1. *Cell Suspensions.* Add 1 full drop of blood to be tested to 1 ml. of isotonic saline solution in a tube to make a 2 per cent cell suspension. Prepare similar suspensions of known rh and Rh_0 cells,

agglutinins. Care and experience are required to determine whether the cell sediment breaks up faster than in the normal control. The minimum titer of anti-Rh typing serum acceptable to the National Institute of Health is 32, as determined by macroscopic observation.

TITRATION OF SERA WITH BLOCKING ANTIBODIES

The titration of anti-Rh serum containing blocking antibodies must be performed against test erythrocytes which are suspended in their own serum, in serum albumin (either human or bovine), or in the serum of AB blood which contains no acquired antibodies. Likewise the serum to be tested must be diluted with one of these protein solutions so that no electrolyte is added to the serum-cell mixture. The cell suspensions are made up in 2 per cent concentration and the procedure is otherwise like that described on pages 135 and 159. The National Institute of Health has established a minimum acceptable titer of 32, as determined by macroscopic examination.

AVIDITY OF SERA WITH BLOCKING ANTIBODIES

Anti-Rh sera with blocking antibodies are frequently employed in slide tests and therefore the avidity of the sera is of some importance. A 40 per cent suspension of erythrocytes of suitable Rh subtype is prepared in serum from the same blood, in bovine or human serum albumin, or in the serum of group AB blood which contains no acquired antibodies. One drop of the cell suspension is placed in the center of a microscope slide, near the drop of serum to be tested. The two drops are mixed with an applicator or wooden toothpick to form an area about 25 mm. in diameter. The slide is held over an electric lamp which not only furnishes illumination but also warms the mixture to a temperature not exceeding 47° C. The slide is tilted continuously but slowly from side to side for three minutes. The time of beginning mixing and the appearance of macroscopic agglutination is accurately measured with a stop watch. The National Institute of Health stipulates that the avidity time is satisfactory when visible agglutination occurs within sixty seconds and some agglutinates are at least 1 mm. in diameter at the end of three minutes.

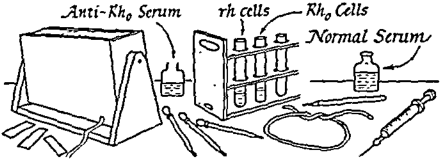
Differentiation Between Rh-Positive and Rh-Negative Blood

In routine transfusions it is generally sufficient to determine whether the blood of donor and recipient is Rh positive or Rh

OPEN SLIDE TEST FOR RH TYPING (Diamond and Abelson[10])

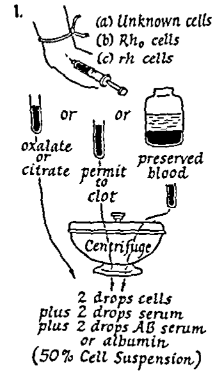
This method is preferred by many workers because it is quick and does not require the use of a water bath. Typing sera may be employed which contain blocking antibodies because the latter act as agglutinins under the conditions of the test.

Equipment and Materials. Serologic test tubes. Microscope slides. Microscope. Drop pipettes. 25-watt electric lamp. Anti-Rh_0 typing serum. Erythrocytes known to belong to subtypes rh and



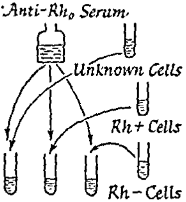
Rh_0. Normal human serum albumin, bovine albumin, serum from the blood of the red cells, or serum from AB blood. Wax pencil. Sterile syringe, needle, tourniquet, sponges, and alcohol.

Procedure



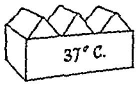
1. *Cell Suspensions.* Prepare oxalated or citrated blood specimens of the unknown type as well as of types rh and Rh_0. *Alternate Method.* Collect blood specimens without anticoagulant and permit them to clot. After withdrawing the cell-free serum, agitate the clot with a glass rod, applicator, or the tip of a pipette, to free as many cells as possible. Remove the clot and centrifuge the serum containing the loose cells. In another tube place 2 drops of the serum from the blood to be tested. Add 2 drops of packed red cells from that blood, making a 50 per cent cell suspension. *Alternate Method.* If the blood to be tested is mixed with a dilute preservative solution, centrifuge the blood mixture. In another tube place 2 drops of human or bovine albumin, or serum from AB blood. Add 2 drops of packed erythrocytes, for a 50 per cent suspension.

2.



2. *Serum-Cell Mixtures.* Label a tube X and add to it 1 drop of the unknown cell suspension. To this add 1 drop of anti-Rh_0 typing serum. Using the same serum, prepare tubes containing rh cells and Rh_0 cells, labeling them appropriately.

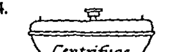
3.



Incubate 30-60 min.

3. *Incubation.* Place the tubes in a water bath at 37° C. for sixty minutes.

4.



4. *Centrifugation.* Shake the tubes and centrifuge for one minute at 500 to 2000 revolutions per minute.

5.



5. *Observation.* Remove the tubes from the centrifuge carefully, one at a time, hold them in the proper illumination and slowly tilt and slightly roll them, watching the cell mass break up for signs of agglutination. The tubes may be held horizontally and examined with the low power objective of a microscope, or the serum-cell mixture may be poured on a slide for microscopic examination.

Interpretation. If the unknown cells and the Rh_0 erythrocytes are agglutinated, but the rh cells are not, the unknown cells may be considered Rh positive (excluding rh′, rh″, and rh′rh″). If no agglutination occurs in the unknown cells but the Rh_0 cells are clumped, the former are considered Rh negative.

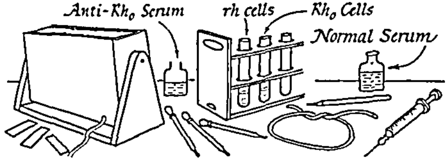
Sources of Error. *Causes of false negative reactions* are (1) mislabeling, (2) weak typing sera, (3) insufficient time of incubation, and (4) too vigorous agitation before reading the results. *Causes of false positive reactions* are (1) mislabeling, and (2) misinterpretation of the appearance of the cell mass.

Checking. There are no complete checks in the method itself. Repetition of the tests with the same serum and cell suspensions may detect some errors in labeling and interpretation of agglutination. Performance of the tests using other antisera and more controls of cells of known subtypes often reveals inconsistencies.

OPEN SLIDE TEST FOR RH TYPING (Diamond and Abelson[10])

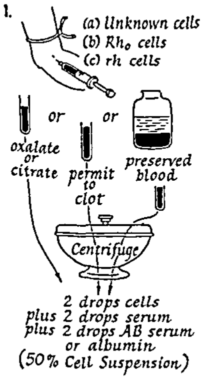
This method is preferred by many workers because it is quick and does not require the use of a water bath. Typing sera may be employed which contain blocking antibodies because the latter act as agglutinins under the conditions of the test.

Equipment and Materials. Serologic test tubes. Microscope slides. Microscope. Drop pipettes. 25-watt electric lamp. Anti-Rh_0 typing serum. Erythrocytes known to belong to subtypes rh and

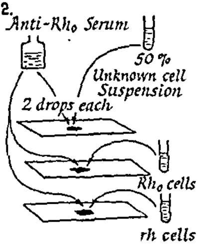


Rh_0. Normal human serum albumin, bovine albumin, serum from the blood of the red cells, or serum from AB blood. Wax pencil. Sterile syringe, needle, tourniquet, sponges, and alcohol.

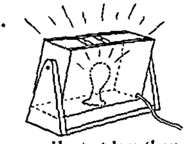
Procedure



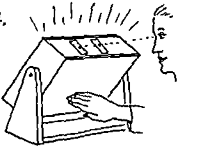
1. *Cell Suspensions.* Prepare oxalated or citrated blood specimens of the unknown type as well as of types rh and Rh_0. *Alternate Method.* Collect blood specimens without anticoagulant and permit them to clot. After withdrawing the cell-free serum, agitate the clot with a glass rod, applicator, or the tip of a pipette, to free as many cells as possible. Remove the clot and centrifuge the serum containing the loose cells. In another tube place 2 drops of the serum from the blood to be tested. Add 2 drops of packed red cells from that blood, making a 50 per cent cell suspension. *Alternate Method.* If the blood to be tested is mixed with a dilute preservative solution, centrifuge the blood mixture. In another tube place 2 drops of human or bovine albumin, or serum from AB blood. Add 2 drops of packed erythrocytes, for a 50 per cent suspension.

2.


2. *Serum-Cell Mixture.* On a slide place 2 drops of anti-Rh_0 serum and add to it 2 drops of the suspension of unknown cells. Make similar preparations of serum and the cells rh and Rh_0.

3.


3. *Incubation and Agitation.* Place the slide preparations on a rack directly over a lighted 25-watt lamp, so that the slides are warmed to a temperature not exceeding 47° C. Tilt the slides gently from side to side occasionally.

4.


4. *Observation.* After three minutes of incubation, observe the slides for evidence of gross or microscopic agglutination.

Interpretation. Agglutination should be present in the Rh_0 cells but not in the rh cells. If the controls react properly, the unknown cells are Rh positive if agglutinated, Rh negative if not.

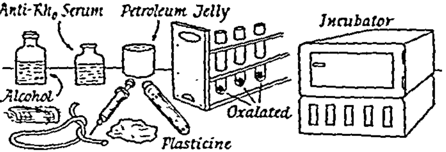
Sources of Error. *Causes of false negative reactions* are (1) mislabeling, (2) weak typing sera, and (3) insufficient heating, agitation, or time of incubation. *Causes of false positive reactions* are (1) mislabeling, (2) nonspecific agglutination, (3) rouleau formation, (4) sedimentation of red cells which is erroneously interpreted as agglutination, and (5) coagulation from excessive heating or from thrombin of serum uniting with fibrinogen in plasma (p. 128).

Checking. Repetition of the tests with the same and different sera should be done. Rouleaux can be identified by microscopic appearance and dissipation of the clumps by the addition of a little saline solution.

CAPILLARY TUBE METHOD FOR RH TYPING (Chown[11])

This test is designed for the use of small amounts of antisera. Blocking antibodies act as agglutinins in this procedure. The requirement for skill and experience is greater in this test than in the centrifuge or slide methods.

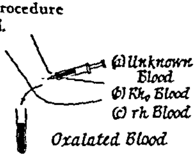
Equipment and Materials. Serologic test tubes. Glass capillary tubes, open at both ends, 0.4 mm. inside diameter and 8 cm. in length, sterilized in test tubes. Plasticine (this is a nonhardening



modeling clay) or petroleum jelly. Anti-Rh_0 serum.. Blood known to be rh and Rh_0. Incubator set at 37° C. Sterile syringes, needles, tourniquet, sponges, and alcohol. Pipettes.

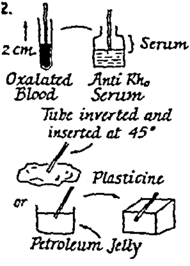
Procedure

1.



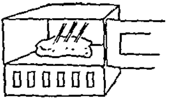
1. *Cell Suspension.* Collect the unknown blood and the control specimens in oxalate or citrate, without further dilution.

2.



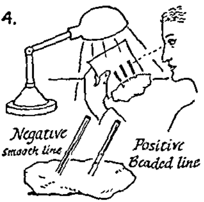
2. *Serum-Cell Mixture.* Dip the end of a capillary tube into the anti-Rh_0 typing serum and permit the reagent to rise in the tube for a distance of 2 cm. Next dip the same end in the blood specimen so that the column of the latter is taken up a similar distance, taking care that no air bubbles are permitted between the two liquids. Invert the tube so that the mixture runs to the opposite end and insert the blood-containing end in a lump of plasticine at an angle of 45°, or dip in petroleum jelly and lean on a rack. Make similar preparations of the known blood specimens.

3.



3. *Incubation.* Place the tubes in the incubator at 37° C. for fifteen minutes.

4.



4. *Observation.* Read by observing the serum-cell column against a white illuminated background. Agglutination causes a beaded layer to form on the lower side of the lumen of the tube, whereas a negative test is indicated by a smooth thin line of red cells forming in the lower side of the lumen.

Interpretation. If the controls react properly, the lack of agglutination in the unknown blood indicates that it is Rh negative; agglutination is evidence that it is Rh positive.

Sources of Error. *Causes of false negative reactions* are (1) mislabeling, (2) weak typing sera, and (3) insufficient time for incubation. *Causes of false positive reactions* are (1) mislabeling, (2) rouleau formation, (3) clotting, and (4) nonspecific agglutination.

Checking. Repetition of the test with the same antiserum and cell suspension will reveal some of the errors. The tests should be performed with other antisera and the use of more controls of known types of Rh.

Determination of the Subtypes of Rh

Occasionally there is need to determine the exact subtype of Rh to which a blood belongs. This is helpful in studying the mechanism of inheritance in families in which there is hemolytic disease of the newborn. Likewise it is an aid in establishing the identity or individuality of blood specimens in medicolegal cases.

Procedure. Any of the methods which are recommended for the differentiation of Rh-positive from Rh-negative blood (p. 161, 163, and 165) are applicable to the determination of the subtypes of Rh. Three typing sera are required: anti-Rh_0, anti-rh′, and anti-rh″. Care should be taken to choose the concentration and method of suspension of the unknown cells which is proper for the method. The cells are suspended in saline solution when a typing serum is employed which contains a blocking antibody

whose action is desired to suppress an agglutinin. On the contrary, if one wishes to employ serum in which the blocking antibody is to be used as an agglutinin, the cells should be suspended in serum albumin or plasma.

Interpretation. The presence or absence of agglutination when the erythrocytes are mixed and incubated with the three antisera determine the subtype of Rh to which the blood belongs:

Rh Subtype of Cells	*Sera* Anti-Rh_0 (85%)	Anti-rh′ (70%)	Anti-rh″ (30%)
rh (Rh negative)	−	−	−
Rh_0	+	−	−
Rh_1 (Rh_0′)	+	+	−
Rh_2 (Rh_0″)	+	−	+
Rh_1Rh_2 (Rh_0′ Rh_0″)	+	+	+
rh′	−	+	−
rh″	−	−	+
rh′ rh″	−	+	+

Sources of Error. The causes of error are the same as those described under the respective methods for differentiating between Rh-positive and Rh-negative blood.

BLOOD TYPING (Hr SYSTEM)

Occasionally it is desirable to determine whether a sample of blood is Hr negative or Hr positive. This is helpful when it is necessary to transfuse a recipient who has become sensitized to the Hr factor. It is possible to determine whether some persons belonging to subtypes Rh_1 and rh′ are heterozygous by demonstrating that they are Hr positive.

Anti-Hr Typing Sera

Sources and Preparation of Sera

Theoretically there are three anti-Hr typing sera, called by Wiener anti-Hr_0, anti-Hr′, and anti-Hr″. The anti-Hr′ is rare, anti-Hr″ has been encountered only once, and anti-Hr_0 exists so far in but few laboratories. The few typing sera so far available have been derived from the blood of human beings who have been sensitized to the Hr factor by pregnancy. No associated blocking antibodies have been demonstrated. The preparation of the typing serum is carried out similar to the procedure for anti-Rh sera (p. 158). Any anti-A or anti-B agglutinins which are present are neutralized by absorption with appropriate red cells or preferably by A and B substance.

METHODS OF STORAGE

The antisera may be kept by the same methods as those employed for anti-Rh sera (p. 159).

TITRATION AND POTENCY OF SERA

The potency of anti-Hr sera usually is low. No standards have been established for minimum titer. Titration is performed in the same manner as for anti-Rh sera (p. 159).

AVIDITY OF SERA

No standards have been established as the minimum acceptable avidity time of these sera.

Differentiation Between Hr Positive and Hr Negative

For practical purposes the terms Hr positive and Hr negative apply to the reaction of cells to anti-Hr′ agglutinins.

CENTRIFUGE METHOD OF HR TYPING

This method is preferred by some to the slide procedure because it is believed to be slightly more dependable in dealing with weak sera.

Procedure. The method is similar to that employed with the anti-Rh sera (p. 160) except that typing sera of anti-Hr′, anti-Hr″, or anti-Hr_0 specificity are used. Of these only anti-Hr′ serum is easily procurable. In employing anti-Hr′ serum control cell suspensions are used belonging to Orh (which is always Hr positive) and ORh_1 *which has been demonstrated to be Hr negative.* The cells are suspended in isotonic saline solution.

Interpretation. Before transfusion of an Hr-negative recipient it is only necessary that the blood of the prospective donor belong to the proper group and be Hr negative. To determine whether the genotvpe of the husband is heterozygous or homozygous the Rh types must be determined with the antisera anti-Rh_0, anti-rh″. The reactions of the cells belonging to rh′ and Rh_1 with anti-Hr′ serum will demonstrate whether the individual is homozygous or heterozygous. With all three Hr antisera available it is theoretically possible to determine the formula of the genotype according to Wiener's theory. With only anti-Hr′ serum the types may be divided into subtypes which have names denoting the presence or absence of the gene *r*. The following table is slightly expanded from Wiener[12]:

Phenotype		Genotype	Antisera			Distribution in Caucasians	
Type	Subtype		Anti-Hr_0	Anti-Hr′	Anti-Hr″		
rh		*rr*	+	+	+		13.0%
rh′	rh′rh′	*r′r′*	+	−	+	0.01	1.01
	rh′rh	*r′r*	+	+	+	1.0	
rh″	rh″rh″	*r″r″*	+	+	−	0 005	0.505
	rh″rh	*r″r*	+	+	+	0.5	
rh′rh″		*r′r″*	+	+	+		0.01
Rh_0		R^0R^0	−	+	+		2 0
		R^0r	+	+	+		
Rh_1	Rh_1Rh_1	R^1R^1	−	−	+	20.0	54.0
		R^1r'	+	−	+		
	Rh_1rh	R^1r	+	+	+	34.0	
		R^1R^0	−	+	+		
		$r'R^0$	+	+	+		
Rh_2	Rh_2Rh_2	R^2R^2	−	+	−	3.0	15.0
		R^2r''	+	+	−		
	Rh_2rh	R^2r	+	+	+	12.0	
		R^2R^0	−	+	+		
		$r''R^0$	+	+	+		
Rh_1Rh_2		R^1R^2	−	+	+		14.5
		$r'R^2$	+	+	+		
		R^1r''	+	+	+		

TESTS FOR ISOSENSITIZATION

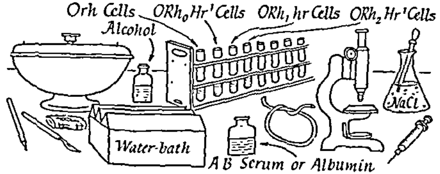
Serologically isosensitization can be demonstrated by (*a*) the finding of specific acquired antibodies (agglutinins or blocking antibodies) in the serum of the sensitized person, or (*b*) in the case of hemolytic diseases of the newborn, by proving that the erythrocytes of the child are sensitized by the acquired antibodies of the mother.

Qualitative Demonstration of Acquired Antibodies

Combined Test for Agglutinins and Blocking Antibodies

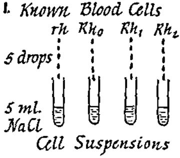
This procedure will demonstrate most of the anti-Rh and anti-Hr antibodies.

Equipment and Materials. Serologic test tubes. Isotonic saline

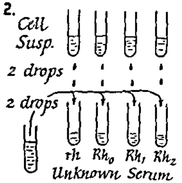


solution. Drop pipettes. Test erythrocytes of the following groups and types: OrhHr′, ORh_0Hr', ORh_1hr, and ORh_2Hr'. Serum from AB blood or 20 per cent serum albumin (bovine or human). Microscope. Water bath set at 37° C. Centrifuge. Wax pencil. Sterile syringe, needle, tourniquet, sponges, alcohol, lancet.

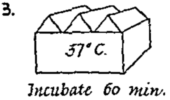
Procedure



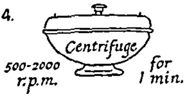
1. *Cell Suspensions.* Place 5 ml. of isotonic saline solution in each of four tubes, labeling them *rh*, Rh_0, Rh_1, and Rh_2, respectively. To each tube add 5 full drops of blood of the type indicated by the label, making 2 per cent suspensions in saline. From each tube transfer 2 drops of cell suspension to another tube, suitably labeled.



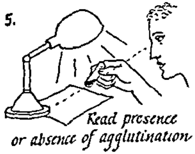
2. *Serum-Cell Mixtures.* Collect about 5 ml. of blood from the person suspected of being sensitized, permit it to clot, and separate the serum. Add 2 drops of the unknown serum to each of the four tubes of cell suspensions.



3. *Incubation.* Place the serum-cell mixture in the water bath at 37° C. for thirty to sixty minutes.



4. *Centrifugation* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.



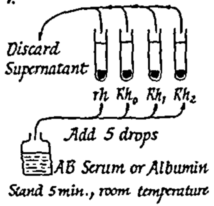
5. *First Observation.* Carefully remove the tubes from the centrifuge, one at a time, and gently tilt and roll slightly under proper illumination, looking for signs of agglutination.

6.



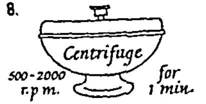
6. *Second Centrifugation.* Recentrifuge the tubes for one minute at 500 to 2000 revolutions per minute.

7.



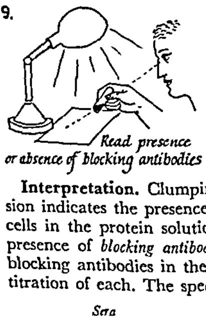
7. *Addition of Protein Solution.* Carefully pipette off the supernatant fluid and discard. Then add 5 drops of AB serum or serum albumin to the packed cells in each tube. Permit the tubes to stand for five minutes at room temperature. It is preferable to incubate for sixty minutes at 37° C.

8.



8. *Third Centrifugation.* Mix the contents of the tubes and centrifuge for one minute at 500 to 2000 revolutions per minute.

9.



9. *Second Observation.* Carefully remove the tubes from the centrifuge, one at a time, gently tilt and roll under proper illumination and look for signs of agglutination.

Interpretation. Clumping of the erythrocytes in saline suspension indicates the presence of *agglutinins.* Agglutination of the red cells in the protein solution, but not in the saline, indicates the presence of *blocking antibodies.* A combination of agglutinins and blocking antibodies in the same serum must be demonstrated by titration of each. The specificity is as follows:

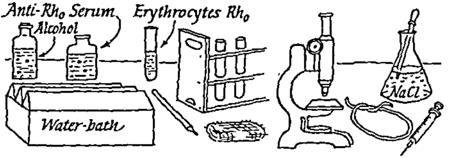
Sera	*Cells*			
	OrhHr′	ORh_0Hr'	ORh_1hr	ORh_2Hr'
Anti-Rh_0', Anti-Rh_0'', Anti-Rh_0	−	+	+	+
Anti-rh′	−	−	+	−
Anti-rh″	−	−	−	+
Anti-Hr′	+	+	−	+

The sera anti-Rh_0', anti-Rh_0'', and anti-Rh_0 can be differentiated only by their reaction to the cells of the rare subtypes rh′

Blocking Test (Wiener[13])

This procedure is employed to demonstrate directly the presence of blocking antibodies in a serum.

Equipment and Materials. Serologic test tubes. Microscope. Wax pencil. Anti-Rh_0 typing serum. Test erythrocytes of type Rh_0.



Water bath set at 37° C. Isotonic saline solution. Sterile syringe, needles, tourniquet, sponges, and alcohol. Pipettes.

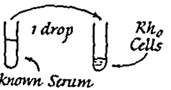
Procedure

1.



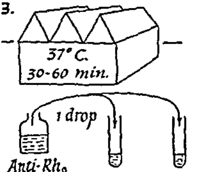
1. *Cell Suspension.* Add 1 full drop of Rh_0 blood to 1 ml. of saline solution in a test tube, making a 2 per cent. cell suspension.

2.



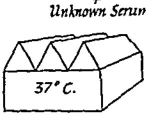
2. *Serum-Cell Mixture.* Collect 1 to 5 ml. of blood to be tested and permit it to clot; separate the serum from the cells. In a tube place 1 drop of the 2 per cent cell suspension and add 1 drop of the unknown serum.

3.



3. *First Incubation.* Place the tube in a water bath at 37° C. for thirty to sixty minutes. After incubation add to the tube 1 drop of anti-Rh_0 typing serum. As a control, place 1 drop of the same typing serum in another tube and add 1 drop of the Rh_0 cell suspension (but none of the unknown serum).

4.



4. *Second Incubation.* Place both tubes in the water bath at 37° C. for thirty to sixty minutes

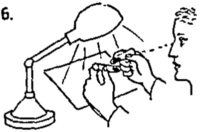
5.



Centrifuge 1 min. 500–2000 r.p.m.

5. *Centrifugation.* Centrifuge both tubes for one minute at 500 to 2000 revolutions per minute.

6.



Agglutination in control = expected
Agglutination in X = no blocking antibodies
No agglutination in X = blocking antibodies

6. *Observation.* Carefully remove the tubes from the centrifuge, one at a time, place in the proper illumination, gently oftilt and roll, and inspect for signs agglutination.

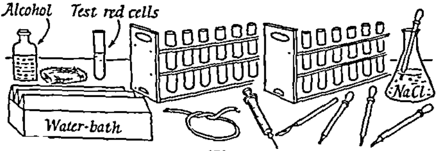
Interpretation. The cells in the control tube should be agglutinated if the typing serum is potent. There should be little or no agglutination in the tube to which the unknown serum is added if blocking antibodies are present in that serum. By making serial dilutions of the unknown serum, this test may be employed to titrate the blocking antibodies.

Titration of Acquired Antibodies

Betore attempt is made to titrate acquired antibodies, the qualitative test described on p. 169 should be performed in order to determine whether agglutinins or blocking antibodies are present. If only agglutinins are present, saline solution is employed to suspend the erythrocytes and to dilute the serum. If blocking antibodies are present, a titration for them should be made in parallel with the test for agglutinins.

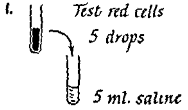
Test for Acquired Agglutinins

Equipment and Materials. Serologic test tubes. Test tube racks Drop pipettes. Water bath set at 37° C. Centrifuge. Wax pencil.

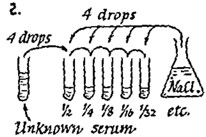


Test erythrocytes of proper specificity. Isotonic saline solution. Sterile syringe, needles, tourniquet, sponges, lancet, and alcohol.

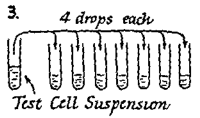
Procedure



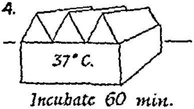
1. *Cell Suspension.* Place 5 ml. of saline solution in a test tube and add to it 5 full drops of blood with the specificity indicated for the titration. This will make a 2 per cent suspension of cells.



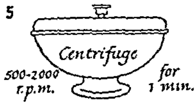
2. *Serum Dilutions.* Collect 2 to 5 ml. of blood from the person suspected of being sensitized and permit the blood to clot. Separate the serum from the clotted erythrocytes. Label a series of tubes with numbers from *1* to *10* inclusive, and add 4 drops of saline solution to each tube. To tube *1* then add 4 drops of the unknown serum, mix, and transfer 4 drops of the mixture to tube *2*, and so on through the series (p. 136).



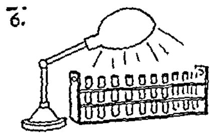
3. *Serum-Cell Mixtures.* To each of the tubes from *1* to *9* inclusive add 4 drops of saline cell suspension, keeping tube *10* in reserve.



4. *Incubation.* Place the tubes in the water bath for sixty minutes at 37° C.



5. *Centrifugation.* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.



6. *Observation.* Carefully remove each tube from the centrifuge separately, place under proper illumination and gently tilt and roll, inspecting for signs of agglutination. Record the results from each tube.

TITRATION OF AGGLUTININS AND BLOCKING ANTIBODIES (Diamond and Denton[14])

If blocking antibodies are present a titration of these should be made.

Equipment and Materials. In addition to the materials and equipment listed on p. 173, about 3 ml. of AB serum or 20 per cent serum albumin (bovine or human) is required.

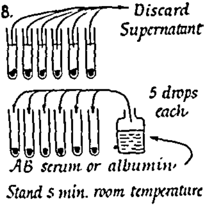
Procedure

7.



1 to **6** *inclusive.* These steps should be carried out as described on pages 173 and 174.

8.



7. *Recentrifugation.* Centrifuge again the tubes containing the serum-cell mixtures. Carefully remove and discard the supernatant serum-saline with clean pipettes.

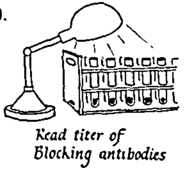
8. *Addition of Albumin or Serum.* Add 5 drops of AB serum or serum albumin to the cell sediment in each tube and permit the mixtures to stand at room temperature for about five minutes. Incubation is probably preferable.

9.



9. *Recentrifugation* Again centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.

10.



10. *Observation.* Again inspect the tubes for the presence or absence of agglutination and record the findings in each tube.

Interpretation. The addition of the inert AB serum or serum albumin to the sensitized erythrocytes results in activating the blocking antibodies so that they cause agglutination.

ALTERNATE TEST FOR BLOCKING ANTIBODIES (Wiener[15])

This is essentially similar to the test for agglutinins (p. 173) with the exception that in addition a parallel series of serum dilutions is used in which the diluent for the serum and the suspending fluid for the test erythrocytes is serum derived from the blood of the test erythrocytes or from AB blood. The titer of the series in which saline solution is employed as serum diluent and as suspending fluid for the cells is taken as an indication of the potency of the agglutinins whereas the titer obtained in the series in which the serum is diluted by serum and the cells are suspended in serum indicates the potency of the blocking antibodies.

Tests for Isosensitized Erythrocytes

Until recently the only serologic proof of isosensitivity was the demonstration in the serum of the sensitized person of agglutinins or blocking antibodies which act against the antigen. Occasionally such proof is difficult to obtain because of the presence of blocking antibodies of high titer. This is particularly true in some cases of erythroblastosis fetalis in which the clinical manifestations of isosensitization may be unequivocal but no antibodies can be demonstrated directly in the laboratory. Several methods are now available, however, by which the erythrocytes of the fetus may be tested directly for evidence of isosensitivity.

ANTI-HUMAN GLOBULIN METHOD FOR SENSITIZED ERYTHROCYTES (Coombs, Mourant, Race[16])

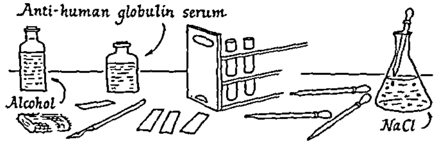
This procedure is a direct test for the antibody absorbed on the surface of the erythrocytes. In a series of cases of erythroblastosis fetalis the authors of the method reported that sensitization occasionally was proved by this maneuver when no agglutinins or blocking antibodies were demonstrable in the serum of the mother. The test has also been employed by Boorman, Dodd, and Loutit[17] to distinguish between congenital and acquired hemolytic icterus. They reported that in five cases of the acquired type the washed erythrocytes were agglutinated by the anti-human globulin serum, whereas negative results were obtained when the red cells from seventeen patients with the congenital type were similarly tested.

Preparation of Antiserum. The anti-human globulin serum is prepared by the injection of rabbits with whole human serum from group O blood, to avoid the possibility of inclusion of the

A and B substances which would also serve as antigens. The procedure is essentially the same as in the preparation of precipitin serum. Two alternate plans are recommended by Kolmer and Boerner.[18] Human serum may be injected intravenously into rabbits at three day intervals in the following dosages: 1, 2, 3, 4, 8, and 10 ml. The other method is the injection intravenously at five day intervals of the following doses: two of 8 ml., two of 5 ml., and two of 3 ml. One-half hour before the injection of each of the larger doses 0.2 ml. of serum is injected to avoid anaphylaxis.

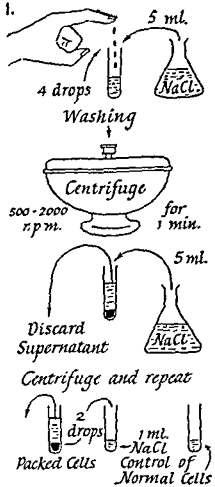
Ten days after the last dose in either series a sample of blood is withdrawn to test the titer. One milliliter of 1/1000 dilution of normal human serum is placed in a serologic test tube and 0.2 ml. of the rabbit immune serum is layered upon it. A cloudy ring of precipitate is searched for at the interface. The presence of precipitins in this test is evidence of sufficiently high titer. If further immunization is indicated, injections of 10, 15, and 20 ml. of human serum are administered intraperitoneally and the antiserum is retested after a ten day rest period. When the titer is sufficiently high, blood is collected from the animals, clotting is permitted, and the serum is separated from the cells.

The serum is inactivated at 56° C. for thirty minutes. Thrice-washed erythrocytes of groups A, B, and O are then added to the serum to absorb the anti-human agglutinins. This procedure is repeated until no agglutinins are demonstrable in less than the dilution at which it is proposed to employ the antiserum. Even a trace of human serum on the cells used for absorption will reduce the anti-globulin titer. The serum should be stored at −15° C. until needed. A portion, diluted to desired strength with saline solution, may be kept for one week at about 0° C.

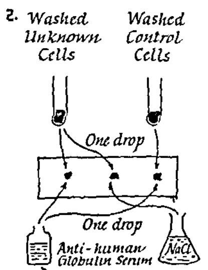


Equipment and Materials. Serologic test tubes. Microscope slides or a white tile. Isotonic saline solution. Drop pipettes. Anti-human globulin serum. Lancet, sponges, and alcohol.

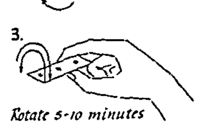
Procedure



1. *Cell Suspension.* Add 4 drops (0.2 ml.) of the whole blood to be tested to 5 ml. of isotonic saline solution, mix, and centrifuge until the cells have sedimented. Discard the supernatant fluid, add fresh saline, and repeat the procedure. After a third washing, prepare a 2 to 5 per cent cell suspension of the cells by adding 2 drops of packed red cells to 1 ml. of saline solution in a test tube. Make a similar suspension of normal erythrocytes as a control.

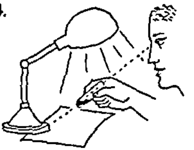


2. *Serum-Cell Mixture.* Place a drop of the cell suspension on a slide or tile and add to it a drop of the anti-human globulin serum. As controls, make a second mixture of 1 drop of the test cells and 1 drop of saline solution; a third preparation is made with 1 drop of the suspension of normal cells and 1 drop of the antiserum.



3. *Agitation.* Tilt the slides or the tile back and forth for from five to ten minutes.

4.



4. *Observation.* Note the presence or absence of agglutination in the test preparation. The controls should not react.

Alternate Procedure. The tests may be performed in test tubes with centrifugation, instead of employing slides or tiles.

Interpretation. If it is shown that the unknown erythrocytes are agglutinated by the anti-human globulin serum, with adequate controls, it is evidence that the cells have been sensitized by an antibody other than anti-A or anti-B agglutinins. The absence of agglutination indicates that sensitization has not occurred.

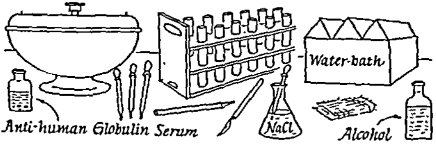
Sources of Error. *Causes of false negative reactions are:* (1) weak antiserum, (2) lowered titer of the antiserum by the presence of traces of human serum with imperfectly washed erythrocytes, (3) insufficient concentration of test cells, and (4) insufficient time allowed for the reaction on the slides or tiles. *A cause of false positive reactions is*: (1) the presence of species-specific anti-human agglutinins in anti-human globulin serum which has been improperly absorbed.

Developing Test (Hill and Haberman[19])

In this method the procedure of Coombs, Mourant, and Race has been somewhat refined and extended. The test is employed either to detect sensitization of erythrocytes directly or to demonstrate the presence of antibodies in low titer which have sensitized erythrocytes of known specific type but have not caused them to agglutinate.

Preparation of Antiserum. The making of anti-human globulin serum is discussed on p. 176.

Equipment and Materials. Serologic test tubes and racks. Drop pipettes. Anti-human globulin serum. A centrifuge. Water bath



adjusted to 37° C. Isotonic saline solution. Wax pencil. Lancet, sponges, and alcohol.

Procedure for Demonstrating Nonspecific Isosensitization

1. *Cell Suspension.* Add 5 drops of whole blood containing the cells to be tested to 5 ml. of saline solution in a test tube. Centrifuge the suspension until the cells are sedimented, discard the supernatant fluid and replace with fresh saline. Repeat the washing twice more. Add 2 drops of the packed cells to 1 ml. of saline in a test tube, making a 2 to 5 per cent cell suspension. Make a similar preparation of erythrocytes which are known not to be sensitized.

2. *Serum-Cell Mixture.* Place 1 drop of the unknown cell suspension in a test tube and add 1 drop of anti-human globulin serum. Make a similar mixture with the serum, using the suspension of normal cells.

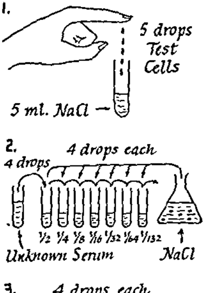
3. *Incubation.* Place the tubes in the water bath at 37° C. for sixty minutes.

4. *Centrifugation.* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.

5. *Observation.* Carefully remove the tubes from the centrifuge, one by one, gently tilt and roll, inspecting them for signs of agglutination.

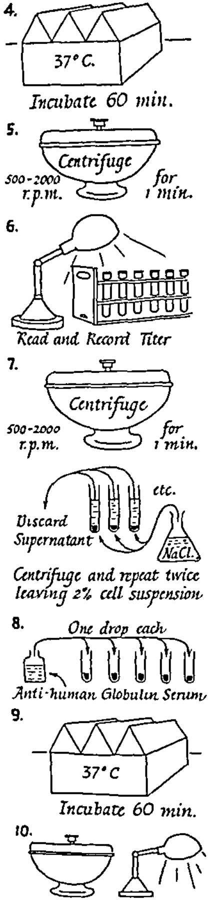
Interpretation. If the unknown cells are agglutinated, the control remaining negative, the erythrocytes have absorbed antibody and are therefore sensitized. There is no indication from this test as to the specificity of the antibodies except that they are not anti-A or anti-B.

Procedure for Demonstrating Specific Antibodies



1. *Cell Suspension.* Select blood belonging to the type whose cells would be expected to contain the antigen specific for the suspected antibodies. For anti-Rh_0 antibodies, use ORh_0 cells; for anti-Hr antibodies, Orh′ cells, and so on. Add 5 full drops of the blood to 5 ml. of saline solution in a test tube, making a 2 per cent suspension.

2. *Serum Dilution.* Label test tubes with the numbers *1* to *10* inclusive, and place in order in the front row of a rack. Fill the back row with unlabeled tubes. Put about 5 ml. of saline solution in each tube in the back row. Add 4 drops of saline solution to each tube in the front row. To tube *1* add 4 drops of the unknown serum, and transfer 4 drops of the mixture to tube *2* after washing the pipette in the saline in the back tube. Make serial dilutions of the serum as described on page 136.



3. *Serum-Cell Mixtures.* Add 4 drops of the cell suspension to each tube containing a serum dilution, and mix.

4. *Incubation.* Incubate the tubes in the water bath at 37° C. for sixty minutes.

5. *Centrifugation.* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.

6. *First Observation.* Carefully remove the tubes from the centrifuge, one at a time, gently tilt and roll while inspecting for agglutination under proper illumination. Record the titer.

7. *Washing of Cells.* Fill each tube containing the serum-cell mixture with isotonic saline solution, mix, and centrifuge until the cells have sedimented. Pipette off and discard the supernatant fluid, replace with fresh saline and wash again. After a third washing remove all but 4 drops of the supernatant fluid, leaving a 2 per cent suspension of sedimented cells. Transfer a drop of each cell suspension to another tube, properly labeled.

8. *Addition of Antiserum.* Add 1 drop of anti-human globulin serum to each tube containing 1 drop of the washed cell suspension.

9. *Reincubation.* Place the tubes in the water bath at 37° C. for sixty minutes.

10. *Repeat Steps 5 and 6.* Record the titer obtained by the second stage of the test.

Interpretation. If the addition of anti-human globulin serum increases the titer or demonstrates activity of antibodies not evident in the first stage of the test, it can be concluded that antibodies specific for the test erythrocytes are present. These are presumably blocking antibodies although the authors of the test believe that a third type of antibody may be demonstrated at times. The titer of the serum is indicated.

Sources of Error. *Causes of false negative reactions are:* (1) weak anti-human globulin serum, (2) the presence of traces of human serum from imperfect washing of the erythrocytes, and (3) insufficient concentration of red cells. *A cause of false positive reactions* is the fact that the anti-human globulin serum may contain anti-human agglutinins which have been imperfectly absorbed.

CROSSMATCHING

When the elements of two blood specimens are mixed, with suitable precautions, to determine whether incompatible antibodies are present, the test is called *crossmatching.* The procedure is employed principally in establishing the compatibility of the blood of donor and recipient before transfusion. The test occasionally proves useful in demonstrating that the maternal serum contains antibodies which react against the cells of the offspring with hemolytic disease of the newborn.

Except in extreme emergencies, crossmatching of the donor's and recipient's blood always should be performed prior to transfusion. To transfuse a patient with blood of homologous group without crossmatching is to ignore the possibility of the presence in the recipient's serum of irregular natural agglutinins, and acquired agglutinins and blocking antibodies. Crossmatching before transfusion furnishes an added valuable check on the accuracy of the blood grouping.

Crossmatching in the A-B-O System

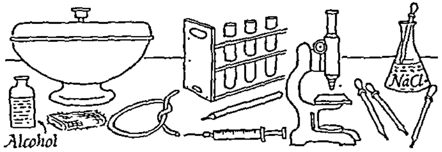
These procedures are intended to demonstrate the presence only of the incompatible natural agglutinins and hemolysins anti-A and anti-B and some of the irregular agglutinins anti-A_1 and anti-O (anti-A_2). These antibodies are characterized by the promptness with which they unite with their specific agglutinogens at room temperature (p. 120). The techniques are designed accordingly. It is important to realize that *these tests will not demonstrate most acquired agglutinins or blocking antibodies.* In employing

only these tests prior to transfusion the presence of dangerously incompatible acquired antibodies will be overlooked in most of the few instances in which they occur.

CENTRIFUGE METHOD OF CROSSMATCHING (A-B-O)

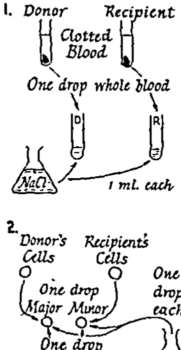
This method is preferable because of its speed and the fact that the agglutinates are definite and large, even when the potency of the antibodies is weak.

Equipment and Materials. Serologic test tubes. Drop pipettes.



Isotonic saline solution. Centrifuge. Microscope. Sterile syringes, needles, tourniquet, sponges, and alcohol. Wax pencil.

Procedure



1. *Cell Suspension.* Collect blood from the prospective donor and recipient. The blood specimens may be permitted to clot or they may be placed in tubes containing oxalate or citrate. Label a tube *D* (for donor) and another *R* (for recipient). Place 1 ml. of saline solution in each tube. Add 1 full drop of the appropriate whole blood to the proper tube, making approximately a 2 per cent cell suspension.



2. *Serum-Cell Mixtures.* Label one tube *major* (for major crossmatch) and another *minor* (for minor crossmatch). To each tube add 1 drop of saline solution. Combine the blood elements as follows:

Major: 1 drop of recipient's serum + 1 drop of donor's cells

Minor: 1 drop of donor's serum + 1 drop of recipient's cells

Interpretation. If the addition of anti-human globulin serum increases the titer or demonstrates activity of antibodies not evident in the first stage of the test, it can be concluded that antibodies specific for the test erythrocytes are present. These are presumably blocking antibodies although the authors of the test believe that a third type of antibody may be demonstrated at times. The titer of the serum is indicated.

Sources of Error. *Causes of false negative reactions are*: (1) weak anti-human globulin serum, (2) the presence of traces of human serum from imperfect washing of the erythrocytes, and (3) insufficient concentration of red cells. *A cause of false positive reactions* is the fact that the anti-human globulin serum may contain anti-human agglutinins which have been imperfectly absorbed.

CROSSMATCHING

When the elements of two blood specimens are mixed, with suitable precautions, to determine whether incompatible antibodies are present, the test is called *crossmatching*. The procedure is employed principally in establishing the compatibility of the blood of donor and recipient before transfusion. The test occasionally proves useful in demonstrating that the maternal serum contains antibodies which react against the cells of the offspring with hemolytic disease of the newborn.

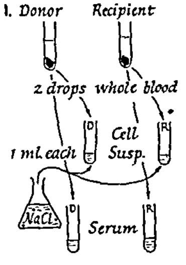
Except in extreme emergencies, crossmatching of the donor's and recipient's blood always should be performed prior to transfusion. To transfuse a patient with blood of homologous group without crossmatching is to ignore the possibility of the presence in the recipient's serum of irregular natural agglutinins, and acquired agglutinins and blocking antibodies. Crossmatching before transfusion furnishes an added valuable check on the accuracy of the blood grouping.

Crossmatching in the A-B-O System

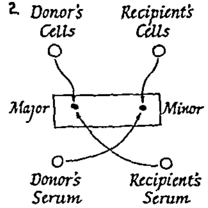
These procedures are intended to demonstrate the presence only of the incompatible natural agglutinins and hemolysins anti-A and anti-B and some of the irregular agglutinins anti-A_1 and anti-O (anti-A_2). These antibodies are characterized by the promptness with which they unite with their specific agglutinogens at room temperature (p. 120). The techniques are designed accordingly. It is important to realize that *these tests will not demonstrate most acquired agglutinins or blocking antibodies*. In employing

tubes. Drop pipettes. Isotonic saline solution. Microscope. Sterile syringes, needles, tourniquet, sponges, and alcohol. Wax pencil.

Procedure



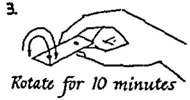
1. *Cell Suspensions.* Collect blood from the prospective donor and recipient. The blood may be permitted to clot or it may be put in tubes containing oxalate or citrate. Label a tube *D* (for donor) and another *R* (for recipient). Place 1 ml. of saline in each tube and add 2 full drops of the whole blood from the proper person to each tube, making a 4 per cent cell suspension.



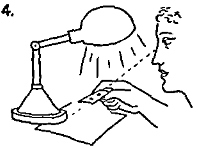
2. *Serum-Cell Mixtures.* Label the left end of a slide *major* (for major crossmatch) and the right end *minor* (for minor crossmatch). Combine the blood elements as follows:

Major: 1 drop of recipient's serum + 1 drop of donor's cells

Minor: 1 drop of donor's serum + 1 drop of recipient's cells



3. *Agitation.* Tilt the slide from side to side continuously for at least ten minutes by the clock. Well slides may be shaken on a mechanical shaker.

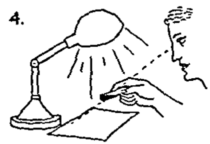


4. *Observation.* The cell-serum mixtures should preferably be observed every minute or so during the period of agitation for signs of agglutination and hemolysis.

Interpretation. Consult page 184 for the interpretation of this procedure.



3. *Centrifugation.* Centrifuge the tubes for one minute at 500 to 2000 revolutions per minute.



4. *Observation.* Remove the tubes promptly from the centrifuge and shake once or twice until the cell mass is broken or disturbed, inspecting for signs of agglutination. Microscopic examination may supplement inspection with the unaided eye.

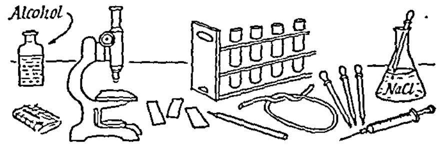
Interpretation. The absence of agglutination in both the major and minor crossmatches indicates that there are no incompatible agglutinins or hemolysins in the A-B-O system. If both blood specimens are thought to belong to the same group but agglutination occurs in the minor match, the blood grouping should be checked. If the bloods do not belong to the same group, then agglutination may be expected in the minor match when certain groups are combined.

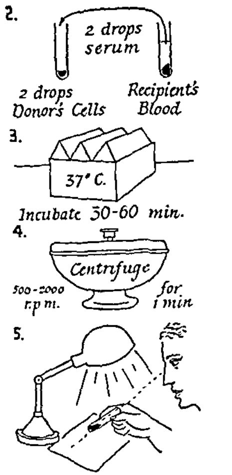
Sources of Error. *Causes of false negative reactions are:* (1) manipulation in which the donor's cells are inadvertently combined with his serum and the recipient's cells with his serum, and (2) the presence of a potent incompatible isohemolysin which destroys the red cells before the agglutinates are observed. To avoid this, the tests should be read immediately after the short period of centrifugation. *Causes of false positive reactions are:* (1) rouleaux, and (2) strong cold hemagglutinins acting at room temperature.

Slide Method for Crossmatching (A-B-O)

This is not recommended for crossmatching if it is possible to employ the centrifuge method, because it is more time-consuming and less reliable in the presence of weak agglutinins.

Equipment and Materials. Plane or well slides. Serologic test





ture, centrifuge about 1 ml. of this and withdraw 1 drop of the packed cells which is added to another tube containing about 1 ml. of serum from AB blood or 20 per cent serum albumin (human or bovine).

2. *Serum-Cell Mixture.* Place 2 drops of the donor's cell suspension in a tube and to this add 2 drops of the recipient's serum.

3. *Incubation.* Place the tube in the water bath at 37° C. for thirty to sixty minutes.

4. *Centrifugation.* Centrifuge the tube for one minute at 500 to 2000 revolutions per minute.

5. *Observation.* Carefully remove the tube from the centrifuge, hold in the proper light and gently tilt and roll, inspecting for signs of agglutination. Microscopic examination should be employed as a check on the naked eye.

Interpretation. Lack of agglutination may be taken as evidence of lack of incompatible agglutinins and antibodies, but not of the absence of isohemolysins.

Sources of Error. *Causes of false negative reactions are*: (1) insufficient time of incubation, (2) breaking up of the agglutinates with overvigorous agitation, (3) overlooking of small agglutinates, (4) the presence of saline solution in serum containing blocking antibodies, and (5) the presence of anti-A or anti-B hemolysins which destroy agglutinates before they have been observed. *Causes of false positive reactions are*: (1) rouleaux, and (2) sedimented erythrocytes mistaken for agglutination.

Open Slide Test for Crossmatching (Rh-Hr) (Diamond and Abelson[20])

This test is rapid and accurate in the hands of the experienced worker. It is somewhat more difficult to read than the centrifuge method because of the heavy cell suspensions.

Equipment and Materials. Serologic test tubes. Microscope slides. Box heated and illuminated with 25-watt electric lamp (p. 163). Drop pipettes. Microscope. Wax pencil. Sterile syringes,

Sources of Error. *Causes of false negative reactions are*: (1) manipulation in which the donor's cells and serum are inadvertently combined and the recipient's cells are tested against the recipient's serum; (2) the presence of a potent isohemolysin which destroys the agglutinates before they are observed; and (3) insufficient agitation in the presence of weak agglutinins. *Causes of false positive reactions are*: (1) rouleaux, (2) strong cold hemagglutinins reacting at room temperature, (3) sedimentation of cells from insufficient shaking, and (4) drying of serum-cell mixtures.

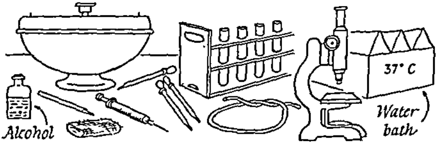
Crossmatching in the Rh-Hr System

The presence of incompatible antibodies with the specificity anti-Rh or anti-Hr require special procedures for their detection. These tests, however, will also demonstrate incompatible anti-A or anti-B agglutinins. The time which is required to perform the maneuvers is so long that if anti-A or anti-B hemolysins are present they might not be detected. It is therefore necessary to perform the rapid crossmatching for the A-B-O system in addition.

CENTRIFUGE METHOD FOR CROSSMATCHING (RH-HR)

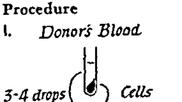
This is preferred by many workers although it probably takes more time than the slide method.

Equipment and Materials. Serologic test tubes. Drop pipettes. Water bath set at 37° C. Centrifuge. Microscope. Wax pencil.



Sterile syringes, needles, tourniquet, sponges, and alcohol. Clotted blood specimen from the recipient.

Procedure



1. *Donor's Cell Suspension.* (1) Collect a specimen of blood from the prospective donor and permit it to clot. Place 0.5 ml. of the donor's serum in a tube. Add to the serum sufficient erythrocytes which have been detached from the clot to make a 2 per cent cell suspension. (2) If the donor's blood has been collected in a preservative mix-

Interpretation. The lack of agglutination may be taken to indicate the absence of incompatible anti-Rh or anti-Hr agglutinins or blocking antibodies.

Sources of Error. *Causes of false negative reactions are*: (1) insufficient warming of the slide, (2) insufficient time of observation, and (3) mistaking small agglutinates for sedimented erythrocytes. *Causes of false positive reactions are*: (1) rouleaux, and (2) mistaking sedimented erythrocytes for agglutinates.

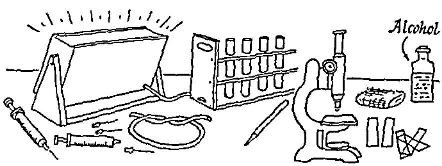
Causes of Agglutination in Crossmatching

When agglutination is encountered in crossmatching the technician is confronted with the problem of determining the cause. Some indications are in order as to the method of approach.

Rouleaux. These may occur either at room temperature or at 37° C. (1) Examine the clumps in the microscope, searching for the characteristic piles of erythrocytes (p. 70). When rouleau formation is intense the typical appearance is not always seen. This is especially true when the cells are derived from preserved blood. (2) Gently dilute the serum of the suspension with an equal quantity of isotonic saline solution. The clumps should disappear or become much smaller with slight mixing. (3) Repeat the original test but use the cells of the same blood, tested against their serum. The serum which produces rouleaux frequently induces the phenomenon in cells from the same blood. (4) Rouleaux are greatest at 37° C. but usually are plainly evident at room temperature. (5) Rouleaux form in a few minutes at room temperature whereas most acquired agglutinins react less readily. (6) Rouleaux can be shaken apart but the agglutinates formed by action of anti-A or anti-B agglutinins are extremely cohesive. The agglutinates formed by anti-Rh and anti-Hr antibodies, on the contrary, are very fragile.

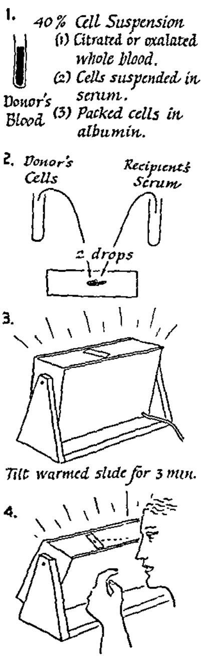
The presence in a serum of rouleau-forming properties usually indicates a high concentration of fibrinogen or serum globulin.

A special problem in rouleau formation is encountered in attempting to crossmatch the blood of a recipient who has received injections of gelatin intravenously. The added colloid produces intense rouleaux which seriously interfere with serologic procedures in which red cells are employed. Vogelaar[21] discovered that the addition of a 1 per cent glycine solution in isotonic saline solution to the serum-cell mixture abolished pseudo-agglutination. In crossmatching with serum containing gelatin Koop and Bullitt advise suspending the cells in 1 per cent sodium citrate in isotonic saline solution. One drop of the donor's cell suspension is added



needles, tourniquet, sponges, and alcohol. Clotted blood specimen from the recipient.

Procedure

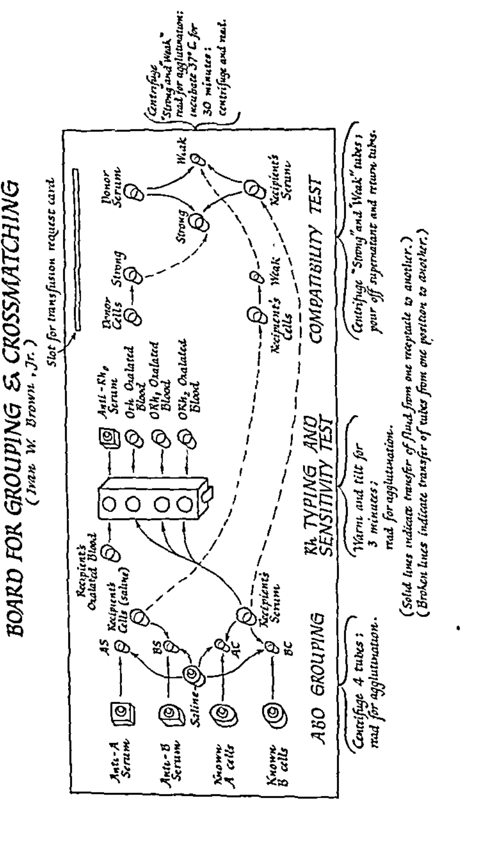


1. *Cell Suspensions.* Prepare a suspension of the prospective donor's cells by one of the three following methods: (1) Collect the donor's blood in a tube containing dry citrate or oxalate (p. 128). Use the whole blood as a suspension. (2) Collect the blood without anticoagulant, permit it to clot, and separate the serum from the cells. Remove 2 drops of the cells from the clot by suction with a pipette and add them to 2 drops of the serum from the same blood, making approximately a 40 per cent suspension. (3) If the donor's blood is in a preservative mixture, centrifuge a sample and add 2 drops of the packed cells to 2 drops of serum from AB blood or serum albumin (human or bovine).

2. *Serum-Cell Mixture.* Place 2 drops of the 40 per cent cell suspension on a microscope slide and add 2 drops of the recipient's serum.

3. *Incubation.* Place the slide over the illuminated box where it will be moderately warmed (optimum 37° C., maximum 47° C.) and tilt back and forth occasionally, for three minutes.

4. *Observation.* Inspect with the unaided eye for signs of agglutination. Microscopic examination is not very helpful because of the heavy concentration of erythrocytes, Rouleaux are prone to occur but can be differentiated by their slightly granular appearance.


BOARD FOR GROUPING & CROSSMATCHING
(Ivan W. Brown, Jr.)
Slot for transfusion request card
Anti-A Serum
Anti-B Serum
Known A cells
Known B cells
Saline
AS
BS
AC
BC
Recipient's Cells (saline)
Recipient's Serum
Recipient's Oxalated Blood
Anti-Rh₀ Serum
Orh Oxalated Blood
ORh₁ Oxalated Blood
ORh₂ Oxalated Blood
Donor Cells
Donor Serum
Recipient's Cells
Recipient's Serum
Strong
Weak
ABO GROUPING
Rh TYPING AND SENSITIVITY TEST
COMPATIBILITY TEST
Centrifuge 4 tubes; read for agglutination.
Warm and tilt for 3 minutes; read for agglutination.
Centrifuge "Strong" and "Weak" tubes; pour off supernatant and return tubes.
Centrifuge "Strong" and "Weak" read for agglutination; incubate 37° C. for 30 minutes; centrifuge and read.
(Solid lines indicate transfer of fluid from one receptacle to another.)
(Broken lines indicate transfer of tubes from one position to another.)

to a drop of 1 per cent glycine in saline, and the mixture is added to a drop of the recipient's serum. The procedure is then carried out in the usual manner.

Anti-A or Anti-B Agglutinins. These are active at either room temperature or 37° C. (1) Carefully repeat the grouping of both blood specimens of the crossmatch. If both bloods belong to group A, test for the irregular agglutinins anti-A_1 or anti-O (anti-A)$_2$ with erythrocytes of appropriate subgroup (p. 55).

Anti-Rh or Anti-Hr Agglutinins. These usually react during incubation at 37° C. They are not likely to be active at room temperature unless they are unusually potent. Test the serum which agglutinates against ORh and Orh cells. If the serum agglutinates Orh cells it contains either an anti-Hr agglutinin or an anti-O antibody. The reaction of the serum to the ORh cells should differentiate; the anti-O will agglutinate both Orh and ORh, the anti-Rh will clump only ORh.

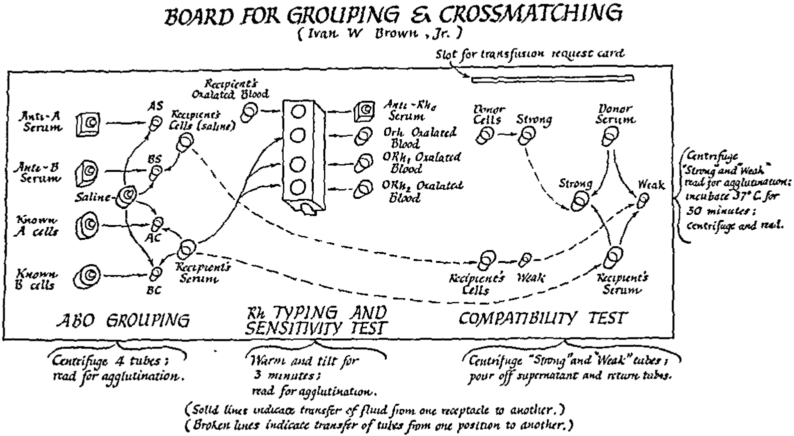
Cold Hemagglutinins. Occasionally cold hemagglutinins in high titer are active at room temperature, rarely at 37° C. Incubation at 37 C. usually causes the clumps from cold agglutinins to break up completely, thus differentiating them from the agglutinates formed by anti-A, anti-B, anti-Rh, and anti-Hr antibodies.

BROWN'S PLAN FOR GROUPING AND CROSSMATCHING

The multiplicity of tests which are now required on the donor's and recipient's blood before transfusion has necessitated some systematization of the laboratory procedures to avoid confusion of the technician. In order to save waste motions and minimize the dangers from human error Brown[22] of Duke University has designed a board to be employed for the grouping and crossmatching of blood. All routine procedures are indicated by appropriate labeling and lines painted on the board. Holes are made for reagent bottles and test tubes. The salient features of the plan are:

Arrangement. The board is divided into three sections, one for blood grouping in the A-B-O system, another for Rh typing and the testing of the recipient's serum for Rh and Hr antibodies, and a third for crossmatching.

Identification of Receptacles. As far as practical, all tubes and reagent bottles bear permanent labels. Each bottle is of distinctive shape and fits into its hole on the board so that it cannot be misplaced. The tubes bear permanent labels and are further differentiated by their size and the holes into which they fit. Serologic tubes, 75 by 10 mm., are labeled *AS* for "Anti-A Serum," *BS* for


BOARD FOR GROUPING & CROSSMATCHING
(Ivan W Brown, Jr.)
Slot for transfusion request card
Anti-A Serum
Anti-B Serum
Saline
Known A cells
Known B cells
AS
BS
AC
BC
Recipient's Cells (saline)
Recipient's Oxalated Blood
Recipient's Serum
Anti-Rh₀ Serum
Orh Oxalated Blood
ORh₁ Oxalated Blood
ORh₂ Oxalated Blood
Donor Cells
Strong
Donor Serum
Strong
Weak
Recipient's Cells
Weak
Recipient's Serum
ABO GROUPING
Rh TYPING AND SENSITIVITY TEST
COMPATIBILITY TEST
Centrifuge "Strong" and "Weak" read for agglutination: incubate 37° C. for 30 minutes; centrifuge and read.
Centrifuge 4 tubes; read for agglutination.
Warm and tilt for 3 minutes; read for agglutination.
Centrifuge "Strong" and "Weak" tubes; pour off supernatant and return tubes.
(Solid lines indicate transfer of fluid from one receptacle to another.)
(Broken lines indicate transfer of tubes from one position to another.)

"Anti-B Serum," *AC* for "Known A Cells," and *BC* for "Known B Cells." Tubes, 75 by 12 mm., are marked *S* for "Strong," indicating the major crossmatch (donor's cells + recipient's serum), whereas smaller tubes (75 by 10 mm.) are marked *W* for "Weak" to designate the minor crossmatch (recipient's cells + donor's serum). Thus a tube for one purpose will not fit into the hole intended for another. Tubes, 100 by 13 mm. are used for recipient's cells, recipient's serum, recipient's oxalated blood, donor's cells, and donor's plasma.

Unity of Procedure. The technician is compelled by this plan to perform all the tests required for grouping, typing, and crossmatching pertaining to a single transfusion before those for another transfusion can be attempted. In a busy laboratory this obviates much of the chance of confusing specimens. Somewhat more time is consumed than if a single test were performed on a rack of specimens at one time, but the danger of confusion is probably much less.

Methods of Testing. A tube of clotted blood and another of oxalated blood is collected from each prospective recipient. Blood grouping is performed by the centrifuge method. Rh typing and tests for antibodies in the recipient's serum utilize the slide method of Diamond and Abelson. A small brass viewing box, illuminated and heated by a 6-watt electric lamp, is mounted on the board so that it can be tilted. A well slide with four wells is placed on the viewing box. The compatibility tests are performed with the centrifuge technique and the tubes are incubated in a water bath. After placing the cell suspensions in tubes *S* and *W* the latter are centrifuged and the supernatant saline solution is poured off. When the serum is added in making the crossmatch the resulting suspension is stated by the author to permit agglutination caused by blocking antibodies.

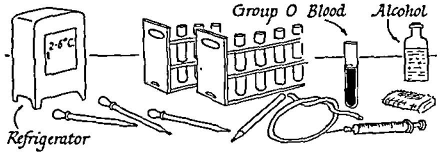
COLD HEMAGGLUTINATION

The demonstration of cold hemagglutinins in high titer in the serum of patients is occasionally helpful in the diagnosis of virus pneumonia, hemolytic anemias, and peripheral vascular disease. Cold agglutinins are present in low titer in most normal persons. The antibodies are not specific for blood group or type but they react with the erythrocytes of most human beings as well as those of certain other animals. They are readily absorbed at low temperatures by the erythrocytes of the blood from which they are derived so that precaution must be employed in controlling the temperature during collection of the blood and the performance of the tests.

Titration of Cold Hemagglutinins

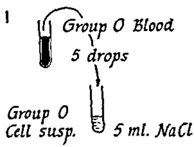
Centrifuge Method of Titration (Cold Agglutinins)

Equipment and Materials. Serologic test tubes. Test tube racks. Drop pipettes. Isotonic saline solution (0.9 per cent NaCl).

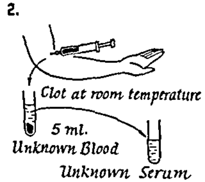


Group O blood. Refrigerator set at 2° to 6° C. Water bath set at 37° C. Wax pencil. Sterile syringes, needles, tourniquet, sponges, and alcohol.

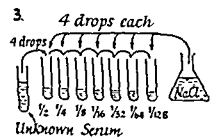
Procedure



1. *Cell Suspension.* Make a 2 per cent cell suspension by adding 5 full drops of group O blood to 5 ml. of isotonic saline solution in a tube.

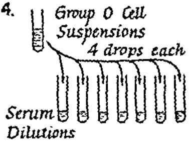


2. *Preparation of Test Serum.* Collect about 5 ml. of blood to be tested in a dry syringe and place in a tube. Permit it to clot *at room temperature* (so that the cells will not absorb the cold hemagglutinins). Centrifuge the clot down and remove the cell-free serum.



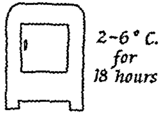
3. *Serum Dilution.* Label ten tubes with the numbers *1* to *10*, inclusive, and place in the front row of a rack. Place unlabeled tubes in the back row. In each tube of the front row place 4 drops of saline and in each tube of the back row put about 5 ml. To tube *1* add 4 drops of the serum to be tested, mix, and transfer 4 drops of the mixture to tube *2*, and so on throughout the series, washing the pipette in the saline of the back tube after each transfer. The dilutions will be ½, ¼, ⅛, etc. (p 136).

4.



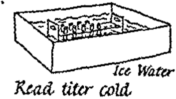
4. *Serum-Cell Mixture.* Add 4 drops of the cell suspension to each of the first nine tubes of the diluted serum, keeping *10* in reserve. Shake the rack to mix the contents of the tubes.

5.



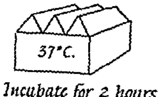
5. *Refrigeration.* Place the rack of tubes in the refrigerator at 2° to 6° C. for about eighteen hours.

6.



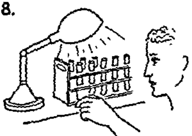
6. *Observation.* Remove the rack of tubes from the refrigerator and immerse in a pan of ice water. Pick up the rack and shake once or twice to resuspend the cell masses. Read the end point of agglutination with the naked eye and record. If agglutination has occurred in all nine tubes, extend the series of serum dilutions, starting with a transfer from tube *10* which has been kept in reserve (p. 137), and repeat the test.

7.



7. *Incubation.* Place the rack of tubes in the water bath at 37° C. for two hours.

8.



8. *Second Observation.* Remove the tubes from the water bath, shake to resuspend the cells, and observe for signs of agglutination.

Interpretation. The titer of cold hemagglutinins is the reciprocal of the greatest dilution of serum which produces agglutination in the cold, as observed macroscopically. All agglutination should disappear when the tubes are subjected to a temperature of 37° C.

STATE OF PRESERVATION OF BLOOD

The object of blood preservation is to maintain the integrity of the erythrocytes. The ultimate test of satisfactory preservation is the measurement of the survival of transfused erythrocytes in

the circulation of the recipient, but this procedure is impractical in routine clinical practice. Some indications can be obtained about the state of the preserved red cells by other tests. If the amount of spontaneous hemolysis during storage is excessive, it may be assumed that the integrity of many erythrocytes yet unruptured is precarious. The osmotic state of the erythrocytes may be tested by immersion in 0.9 per cent sodium chloride solution. If the red cells are found to rupture under these conditions it is probable that they will rapidly disintegrate in the plasma of the recipient.

Amount of Spontaneous Hemolysis

Inspection of Hemolysis

In the operation of a blood bank the worker soon acquires the experience to recognize when the concentration of hemoglobin is excessive in the supernatant plasma of a flask of sedimented preserved blood. The actual intensity of the red color which is imparted to plasma by a certain total amount of free hemoglobin depends upon the dilution of the plasma by the, preservative mixture and the cross section of the container. Simple inspection by a skilled observer is the method employed in routine practice.

Chemical Determination of Hemoglobin (Flink and Watson[23])

In some instances an exact quantitative determination of hemoglobin in the plasma of preserved blood or the recipient with hemoglobinemia is desired. The following method is designed to measure the concentration of hemoglobin and related pigments, sometimes referred to collectively as hemochromogens.

Calibration of the Colorimeter. *Equipment and Materials.* Photoelectric colorimeter. Colorimeter tubes of approximately 10 ml. capacity. A 425 millimicron filter.* Chemically pure pyridine. 10 per cent ammonium hydroxide solution. 0.2 to 0.4 per cent sodium hydrosulfite ($Na_2S_2O_4$) solution, freshly made with dilute ammonia. Three per cent hydrogen peroxide solution. Three 100 ml. volumetric flasks, two 50 ml., and four 25 ml. Volumetric pipettes with capacity of 20 ml., 10 ml., 5 ml., 2 ml., and 1 ml. Serologic pipettes, measuring in 0.01 ml. Sterile 10 ml. syringe, with needles, tourniquet, sponges, and alcohol. One test tube containing dry potassium and ammonium oxalate sufficient for 10 ml. of blood (p. 128). Copper sulfate standards for the determination of hemoglobin concentration (p. 215).

*The original authors used a 550 millimicron filter but we have found that one of 425 millimicrons seems to be necessary.

Dilution of Hemoglobin. Collect about 10 ml. of blood from a normal person and place in a test tube containing as a coagulant dry potassium and ammonium oxalate. Make dilutions of the blood with distilled water according to Table XIII. In measuring out the volumes of blood called for, use volumetric pipettes to the closest full ml. and use the serologic pipettes for the fractions of a ml.

TABLE XIII

Dilutions of Blood Used in Calibration of Colorimeter in Hemochromogen Method

Volume of Blood	Dilute with Distilled Water to:	Dilution Factor	Hemoglobin Concentration Mg. Hb/100 ml. (Calculate: Mg./100 ml. Hb. in whole blood x dilution factor)
4 00 ml whole blood	100 ml.	1/25	—
20.83 1/25 dilution	25	1/30	—
15.62 " "	25	1/40	—
12.50 " "	25	1/50	—
8 33 " "	25	1/75	—
8.33 " "	50	1/150	—
4.17 " "	50	1/300	—
3 33 " "	100	1/750	—
1.67 " "	100	1/1500	—

Reading of Hemoglobin Solutions. Place a 425 millimicron filter in the photoelectric colorimeter. Read each concentration of hemoglobin after it has been treated chemically, before another is begun. Place 1.0 ml. of the known hemoglobin solution in a colorimeter tube and add to it 6.5 ml. of 10 per cent ammonium hydroxide solution. Then add 0.5 ml. of pyridine and 2.0 ml. of sodium hydrosulfite solution. Make a *blank* by placing 1.0 ml. of distilled water in another colorimeter tube, to which add 5.5 ml. of ammonium hydroxide, 0.5 ml. of pyridine, and 2 0 ml. of sodium hydrosulfite. Add to the blank 1 ml. of 3 per cent hydrogen peroxide and permit to decolorize for five minutes. Then place the blank in the colorimeter and adjust accordingly. The tube with the hemoglobin is then placed in the colorimeter and the galvanometer deflection is observed. The galvanometer readings for all the hemoglobin solutions are converted to terms of *optical density* if such readings are not available on the dial. Optical density = 2 − logarithm of per cent transmission.

Hemoglobin Concentration of Original Blood. Determine the hemoglobin concentration of the oxalated blood by the copper sulfate method (p. 215).

Plotting the Curve. When the concentration of hemoglobin has been obtained for the original blood specimen, multiply the value in milligrams by the dilution given in Table XIII for each hemoglobin solution which was tested. Lay off an appropriate scale for hemoglobin concentration in milligrams on the abscissas of a linear graph. On the ordinates construct a scale for values of optical density. Then plot the values observed for the known hemoglobin dilutions, and connect the points with a curve. If the galvanometer dial does not register values in terms of optical density, but only as per cent transmission, semi-log paper may be used for the curve. In the latter case, lay off the linear abscissas as before but the per cent transmission is constructed on the logarithmic scale of the ordinates. The observed values can then be plotted directly.

Equipment and Materials. Photoelectric colorimeter. Colorimeter tubes holding approximately 10 ml. A 425 millimicron filter. Chemically pure pyridine. 10 per cent ammonium hydroxide solution. 0.2 to 0.4 per cent sodium hydrosulfite ($Na_2S_2O_4$) solution, freshly made with dilute ammonia. Three per cent hydrogen peroxide. Calibrated pipettes. Centrifuge. Calibration curve for method. Test tubes. Wax pencil.

Procedure

1. *Preparation of Unknown.* Centrifuge the unknown plasma until it is free from red cells. Place from 0.02 to 1.0 ml. of plasma (depending on the concentration of hemoglobin) in a colorimeter tube and make up to 7.5 ml. with 10 per cent ammonium hydroxide. Then add 0.5 ml. of pyridine and 2.0 ml. of sodium hydrosulfite solution. We have found that the plasma should be added *last.*

2. *Preparation of Blank.* Place the same amount of plasma in another colorimeter tube and make up to 6.5 ml. with ammonium hydroxide. Add 0.5 ml. of pyridine and 2.0 ml. of hydrosulfite solution. Finally add 1 ml. of 3 per cent hydrogen peroxide and permit the mixture to decolorize for five minutes.

3. *Reading.* Place the decolorized blank in the colorimeter and adjust accordingly. Then place the tube with the unknown in the colorimeter and read the galvanometer deflection.

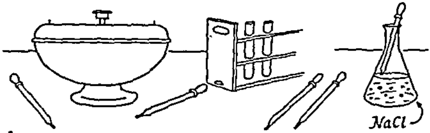
Calculation. With the value obtained from the colorimeter reading obtain the hemoglobin concentration from the curve previously constructed. If the amount of plasma employed in the test is less than 1.0 ml., multiply the concentration obtained on the curve by a fraction having 1 as the numerator and the actual volume used as the denominator. This will give the hemoglobin concentration in milligrams per 100 ml. of plasma.

Osmotic State of the Erythrocytes

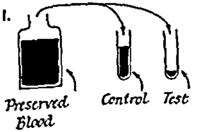
There is considerable variation in the rate of deterioration during storage of red cells from different individuals even when the conditions of storage are similar. The outdating period which is given for various preservative mixtures is for average blood, and exceptions are frequently encountered. The altered osmotic state of the erythrocytes during storage is not always reflected in the amount of spontaneous hemolysis. The plasma mixture may be slightly hypertonic but the contents of the red cells equilibrate with their surrounding fluid. Under such circumstances a flask of blood which appears suitable may be transfused and the red cells be ruptured when they come in contact with the plasma of the recipient. For this reason it is desirable to test each flask of preserved blood before transfusion. For years the authors have employed a simple test which occasionally reveals a flask of preserved blood in which the osmotic fragility of the erythrocytes is such as to make them unsuitable for transfusion. Such blood may be converted to plasma and the erythrocytes discarded.

RAPID TEST FOR OSMOTIC FRAGILITY (DeGowin, Harris, Bell, and Hardin[24])

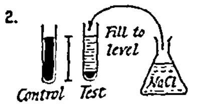
Equipment and Materials. Two serologic test tubes. Pipettes. Centrifuge. Isotonic saline solution (0.9 per cent Na Cl).



Procedure



1. *Control Tube.* Fill one tube nearly full of the blood mixture to be tested.



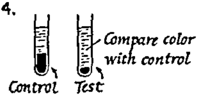
2. *Blood Saline Mixture.* Place approximately 0 5 ml. of the blood mixture in a second tube and fill to the approximate level in the control tube with isotonic saline solution. Mix.

3.



3. *Centrifugation.* Centrifuge the two tubes for one minute at 500 to 2000 revolutions per minute.

4.



4. *Observation.* Compare the intensity of the red color in the supernatant fluid of the two tubes by inspection.

Interpretation. The plasma diluted with saline should be a lighter red than that in the control. If the saline-plasma is as red or redder, erythrocytes have been ruptured by isotonic saline solution and presumably would react similarly in the plasma of the recipient, if transfused.

MEASUREMENT OF SURVIVAL OF TRANSFUSED ERYTHROCYTES

The presence of transfused red cells in the circulation of the recipient may be studied by (1) the enumeration of the inagglutinable erythrocytes of the donor in the blood of the recipient after transfusion of blood of heterologous group or type, and by (2) the measurement of radioactivity in the circulation of the recipient after transfusion of blood from a donor who has incorporated a radioactive isotope of iron in his hemoglobin. A rapid loss of transfused erythrocytes from the circulation of the recipient may be detected by (1) the demonstration of an increased level of hemochromogens and bilirubin in the blood plasma of the recipient soon after transfusion, and by (2) the increase in excretion of fecal urobilinogen after transfusion.

There is good agreement by various methods of measurement that the average red cell survives in the circulation of the normal person about one hundred days. This implies that a sample of blood taken from that person will contain approximately 1 per cent of red cells which are one day old, 1 per cent which are two days old, and so on up to 1 per cent which are one hundred days of age.

One would expect that under the best conditions of transfusion, therefore, that the minimum loss of transfused red cells from the circulation of the recipient would be approximately 1 per cent per day.

Differential Agglutination

In the blood of persons belonging to groups A, B, and AB there are certain erythrocytes which are not clumped by appropriate anti-A or anti-B agglutinin sera. The number of *inagglutinable erythrocytes* is more or less constant for a given grouping serum, and perhaps for the individual. Ashby[25] devised a procedure in which the inagglutinable cell count of the recipient belonging to group A, B, or AB is determined before and after transfusion with a measured amount of group O blood. Any increase in the number of inagglutinable cells in the circulation of the recipient is attributed to the presence of the donor's erythrocytes. Wiener[26] modified the Ashby method by transfusing the recipient with blood of homologous group in the A-B-O system, but the recipient should belong to type M and the donor to type N or *vice versa*. The appropriate anti-M or anti-N serum is employed to identify the donor's cells in the circulation of the recipient.

Tests which employ the principle of agglutination have the advantage of furnishing direct estimations of the number of donor's cells in the recipient's circulation throughout the entire life of the transfused cells. A large quantity of highly potent antiserum is required. Much practice with the method is necessary to obtain reliable results.

The method of differential agglutination has been applied to a variety of problems in clinical medicine. Studies of the survival of freshly drawn erythrocytes from normal persons in the circulation of normal recipients have furnished the best data on the life of normal red cells. The methods of blood preservation may be evaluated by measuring the persistence in the recipient's blood stream of red corpuscles stored in various ways. Hemolytic transfusion reactions sometimes may be elucidated by the agglutination technique. Some hemolytic blood dyscrasias have been investigated by measuring the survival of blood cells from normal persons in patients with the disorders, or by transfusion of the abnormal cells into normal recipients.

The Antiserum. An agglutinating serum of high potency and suitable specificity should be selected for the study. The same lot of serum should be employed in all tests on the recipient during the experiment because the inagglutinable cell count for the individual varies somewhat with sera of different potencies. Since the study may cover a period of over one hundred days, precautions must be taken to insure the stability of the agglutinins. Before commencing the study sufficient antiserum to complete all tests planned should be prepared in small vials, each holding enough for one test. The serum in these should be kept in the frozen state until needed. Some workers have found the use of dry

antiserum extremely satisfactory because of its stability and the fact that its addition to the cells suspensions produces no dilution.

Most workers have found anti-N serum unsuitable for quantitative studies, in contrast to anti-M, anti-A, and anti-B.

Basal Inagglutinable Cell Counts. If anti-A or anti-B serum is employed, daily counts of the inagglutinable cells of the recipient should be made several times before transfusion to obtain a basal level. The average of the values thus determined is subtracted from the inagglutinable cell counts made after transfusion and the difference is considered an enumeration of the donor's cells in the circulation. Agglutinating sera of such potency should be employed which result in basal inagglutinable cell counts of not more than 1 per cent of the recipient's erythrocytes. Osborne and Denstedt have attained inagglutinable cell counts as low as 5000 per cu. mm. by using antisera with a titer of 1/5000 (J. Clin. Investigation **26**:655, 1947).

The nature of the inagglutinable erythrocytes has not been determined. It is confirmed, however, that reticulocytes respond to the action of antisera as well as cells which contain no reticulum.

Posttransfusion Sampling of the Recipient. The transfused blood may require an hour or so to run into the recipient's vein, and during this time some of the donor's cells may be destroyed. After completion of the transfusion another period of time is consumed in the mixing of the cells of donor and recipient before a representative sample of blood is available. Mollison and Young[27] measured the loss of blood soon after transfusion. They found that when relatively fresh blood was transfused, the count of the donor's cells increased for at least twenty-four hours, which suggested that readjustments of the blood volume occurred for at least that length of time. When older blood was transfused, the greatest concentration of donor's cells was found to occur from four to eight hours after injection. This probably means that the loss of donor's cells begins during transfusion and continues at such a rate that the readjustments in the blood volume, which tend to make the values rise, have the effect of producing the peak concentration about four hours after injection. They estimated the expected maximal concentration of donor's cells in the recipient's circulation from the equation:

$$C_R = \frac{V_D \times C_D}{V_R}$$

where C_R = concentration of donor's erythrocytes in the recipient's plasma in cu. mm.; C_D = concentration of erythrocytes in the transfused blood of the donor in cu. mm.; V_D = volume of donor's blood transfused (the volume of the diluent is ignored); V_R = volume of recipient's blood after transfusion. V_R may be calculated from the equation on page 220.

Differential Agglutination

In the blood of persons belonging to groups A, B, and AB there are certain erythrocytes which are not clumped by appropriate anti-A or anti-B agglutinin sera. The number of *inagglutinable erythrocytes* is more or less constant for a given grouping serum, and perhaps for the individual. Ashby[25] devised a procedure in which the inagglutinable cell count of the recipient belonging to group A, B, or AB is determined before and after transfusion with a measured amount of group O blood. Any increase in the number of inagglutinable cells in the circulation of the recipient is attributed to the presence of the donor's erythrocytes. Wiener[26] modified the Ashby method by transfusing the recipient with blood of homologous group in the A-B-O system, but the recipient should belong to type M and the donor to type N or *vice versa*. The appropriate anti-M or anti-N serum is employed to identify the donor's cells in the circulation of the recipient.

Tests which employ the principle of agglutination have the advantage of furnishing direct estimations of the number of donor's cells in the recipient's circulation throughout the entire life of the transfused cells. A large quantity of highly potent antiserum is required. Much practice with the method is necessary to obtain reliable results.

The method of differential agglutination has been applied to a variety of problems in clinical medicine. Studies of the survival of freshly drawn erythrocytes from normal persons in the circulation of normal recipients have furnished the best data on the life of normal red cells. The methods of blood preservation may be evaluated by measuring the persistence in the recipient's blood stream of red corpuscles stored in various ways. Hemolytic transfusion reactions sometimes may be elucidated by the agglutination technique. Some hemolytic blood dyscrasias have been investigated by measuring the survival of blood cells from normal persons in patients with the disorders, or by transfusion of the abnormal cells into normal recipients.

The Antiserum. An agglutinating serum of high potency and suitable specificity should be selected for the study. The same lot of serum should be employed in all tests on the recipient during the experiment because the inagglutinable cell count for the individual varies somewhat with sera of different potencies. Since the study may cover a period of over one hundred days, precautions must be taken to insure the stability of the agglutinins. Before commencing the study sufficient antiserum to complete all tests planned should be prepared in small vials, each holding enough for one test. The serum in these should be kept in the frozen state until needed. Some workers have found the use of dry

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Calculation.

Unagglutinated cells in 1 cu. mm. = (count in 2 squares) × 5 × 200.

Errors of the Method. The authors of the method state that the error is less than 10 per cent. The variation of the inagglutinable cell count with anti-A or anti-B serum in different individuals is given as being between 10,000 and 100,000 per cu. mm. The method has the disadvantage of having many agglutinates in the counting chamber to interfere with enumeration.

AGGLUTINATION METHOD FOR ERYTHROCYTE SURVIVAL (Denstedt[30])

This procedure uses venous blood and dilution in leukocyte pipettes.

Equipment and Materials. One hemacytometer counting chamber and cover glass. Four leukocyte diluting pipettes. Centrifuge. Microscope. Serologic test tubes. Shaking machine for test tubes. Drop pipettes. Potent anti-A, anti-B, anti-M, or anti-N typing serum, as the case demands. Isotonic saline solution (0.9 per cent NaCl). 3.2 per cent solution of sodium citrate. Sterile syringes, needles, tourniquet, sponges, and alcohol.

Procedure

1. *Collection of Blood.* Withdraw 10 ml. of blood from the vein of the subject and transfer it to a tube containing 1 ml. of sodium citrate solution. Draw the citrated blood into a leukocyte pipette to the mark *0.5* and fill to the mark *11* with isotonic saline solution. Do again with a duplicate pipette.

2. *Serum-Cell Mixture.* Expel the contents of the two pipettes into clean dry serologic tubes. From the contents of these tubes fill dry leukocyte pipettes to the mark *1.0* and draw up the agglutinating serum to the mark *11*. Expel the contents of the pipettes into clean dry serologic tubes.

3. *Centrifugation.* Centrifuge the tubes for two minutes at 1000 to 2000 revolutions per minute.

4. *Agitation.* Shake the tubes for forty-five minutes in a machine at 176 vibrations per minute.

5. *Loading the Chambers.* Permit the contents of the tubes to settle for five seconds and take up some of the supernatant fluid with drop pipettes and fill each half of the counting chamber with cell suspensions from a different tube. Permit the cells to settle to the floor of the counting chamber.

6. *Counting.* Count the unagglutinated cells on all nine large squares (1 mm. on the side) of the counting chamber.

Denstedt and his coworkers[23] estimated that when 400 ml. of blood was transfused into an adult the expected maximum count of donor's cells should approximate 400,000 erythrocytes per cu. mm. This value was taken as 100 per cent and when the observed maximum count fell short, the difference was assumed to be due to the loss of transfused red cells during, or within four hours after, transfusion.

The third alternative method of determining the immediate survival of red cells after transfusion is actually to measure the blood volume of the recipient by one of the procedures described elsewhere (pp. 219, 220, and 222) and to ascribe discrepancies between the expected and the observed counts as due to early loss of the donor's cells.

Many workers make the initial corrected count four hours after transfusion and other samples of blood are examined twelve hours, twenty-four hours, three days, six days, and weekly thereafter.

Denstedt *et al.* have presented evidence to show that there is considerable variability between individual recipients as to the rate at which they lose transfused erythrocytes.

AGGLUTINATION METHOD FOR ERYTHROCYTE SURVIVAL (Thalhimer, Taylor, and Shaub[29])

This method utilizes capillary blood from the finger tip and dilution with red cell counting pipettes.

Equipment and Materials. One hemacytometer counting chamber and cover glass. Two erythrocyte diluting pipettes. One shaking machine for blood pipettes. Potent anti-A, anti-B, anti-M, or anti-N typing serum (liquid), as the case demands. Microscope. Lancet, sponges, and alcohol.

Procedure

1. *Collection of Blood.* Pierce the finger of the recipient, after the applied alcohol has been completely evaporated, so that a free flow is obtained. Draw the blood into a red cell pipette to the mark *0.5.* Then dilute by filling the pipette to the mark *101* with agglutinating serum. Repeat the operation with the second pipette so that duplicate samples are obtained

2. *Agitation.* Shake the pipettes in a shaking machine for five minutes and then permit them to stand at room temperature for at least sixty minutes.

3. *Second Agitation.* Again shake the pipettes for five minutes.

4. *Loading the Chambers.* Quickly discard that portion of the diluting serum in the stem of the pipettes, together with a small portion in the bulbs. Fill each half of the counting chamber with the suspension from a different pipette. Permit cells to settle in chamber.

5. *Counting.* Count all the single erythrocytes found in two large squares (1 mm. on a side). Ignore all agglutinated cells. If the counts in the two squares do not coincide sufficiently, repeat procedure.

Calculation.

Unagglutinated cells in 1 cu. mm. = (count in 2 squares) × 5 × 200.

Errors of the Method. The authors of the method state that the error is less than 10 per cent. The variation of the inagglutinable cell count with anti-A or anti-B serum in different individuals is given as being between 10,000 and 100,000 per cu. mm. The method has the disadvantage of having many agglutinates in the counting chamber to interfere with enumeration.

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5. *Loading the Chambers.* Permit the contents of the tubes to settle for five seconds and take up some of the supernatant fluid with drop pipettes and fill each half of the counting chamber with cell suspensions from a different tube. Permit the cells to settle to the floor of the counting chamber.

6. *Counting.* Count the unagglutinated cells on all nine large squares (1 mm. on the side) of the counting chamber.

Calculation.

$$\text{Unagglutinated cells in 1 cu. mm.} = (\text{count in 9 squares}) \times \frac{10}{9} \times 22 \times 11$$

Errors in the Method. This procedure has two advantages over the preceding method, viz., there are less agglutinates to interfere with enumeration of the cells in the chamber, and a smaller amount of typing serum is employed. It requires considerable experience to learn to take up the cell suspensions at the proper time in step 5.

AGGLUTINATION METHOD FOR ERYTHROCYTE SURVIVAL (Young, Platzer, and Rafferty[31])

This procedure was evolved from several methods employing dry antiserum and its accuracy has been carefully evaluated.

Equipment and Materials. Blood sample bottles of about 10 ml. capacity, each containing 4 mg. dry potassium oxalate and 6 mg. dry ammonium oxalate. Serologic pipettes of 1.0 ml. capacity. Capillary pipettes. Volumetric flask of 100 ml. capacity. Serologic test tubes. Dried antiserum of suitable specificity (anti-A, anti-B, or anti-M). Clean wooden toothpicks. Four hemacytometer counting chambers and cover glasses, two microscopes (preferably). Centrifuge. Isotonic saline solution (0.9 per cent NaCl). Wax pencil. Dry syringes, needles, tourniquet, sponges, and alcohol.

Procedure

1. *Collection of Blood.* Draw approximately 5 ml of blood in a syringe from a vein of the subject, with a minimum of stasis, and expel it into a sample bottle containing dry oxalate. Rotate the contents of the bottle gently until the blood is thoroughly mixed with the powder.

2. *Dilution of Blood* Transfer 0.5 ml. of the oxalated blood, carefully measured in a serologic pipette, to the 100 ml. volumetric flash which has been partly filled with saline solution. After mixing, fill the flask to the mark with saline solution and thoroughly mix. This makes a blood dilution of 1/200.

3. *Serum-Cell Mixture.* Label four serologic test tubes with the numbers *1* to *4*, inclusive. In each of tubes *3* and *4* place the amount of dry antiserum which can be held on the broad tip of a toothpick (this will be between 2 and 4 mg). Transfer 0.2 ml. of the 1/200 cell suspension, thoroughly mixed, to each of the four tubes, using a serologic pipette. Shake tubes gently for three minutes.

4. *Centrifugation.* Centrifuge tubes *3* and *4* for one minute at 1000 to 2000 revolutions per minute. Shake the tubes by flicking against the table for fifteen seconds and then let stand at room temperature for five minutes.

5. *Filling the Chambers.* Label the four counting chambers *A*, *B*, *C*, and *D*. Reshake the four tubes for fifteen seconds and then transfer the cell suspensions to the counting chambers with separate capillary pipettes in the following plan: the contents of tube *1* to the left side of chambers *A* and *B*, that from tube *2* to the right side of the same chambers; the mixtures from tube *3* to the left side of chambers C and *D*, while the contents of tube *4* is placed in the right side of the same chambers. Let technician I fill the left side of all four chambers and technician II fill the right side, discarding the pipettes after a single transfer.

6. *Counting.* Let technician I count the cells in chambers *A* and *D* while technician II counts the chambers *B* and C. Count the unagglutinated cells in two patterns each of five small (0.2 mm. on a side) squares on each side of a counting chamber. One pattern consists of the four corner and the central square in one large square (1.0 mm. on a side); the other pattern comprises another five squares adjacent to those of the first pattern. If a square contains a large agglutinate, it is not counted but an adjacent square is enumerated instead. Each technician thus counts four patterns of cells in saline and four of the serum-cell mixture.

Calculation.

$$\text{Average of observed counts of cells in saline} = \frac{\text{sum of counts in 8 patterns}}{8}$$

$$\text{Average of observed counts of cells in serum} = \frac{\text{sum of counts in 8 patterns}}{8}$$

Total red cells per cu mm. = (average count in saline) × 10,000
Donor's red cells per cu. mm. = (average count in serum) × 10,000 − (basal inagglutinable cell count in cu. mm.)
Recipient's red cells per cu. mm. = (total red cells per cu. mm.) − (donor's red cells per cu. mm.)

Errors of the Method. Counts are disregarded and the procedure repeated if either technician suspects a break in technique, or if the counts for the different patterns of the same cell suspension vary by more than 1.5 standard deviations. The standard deviation σ may be calculated from the formula

$$\sigma = \sqrt{\frac{\Sigma(x^2)}{N}}$$

where Σ refers to the sum, x = the deviation of a count from the mean of all the counts, and N = the number of counts.

For example:

Counts in 8 patterns	*Deviations from mean of 493*	*Squares of deviations*
	x	x^2
525	32	1024
575	82	6724
425	68	4624
475	18	324
500	7	49
525	32	1024
450	43	1849
475	18	324
3950		15942

N = 8

$$\text{mean} = \frac{3950}{8} = 493 \qquad \Sigma(x^2) = 15942$$

$$\sigma = \sqrt{\frac{\Sigma(x^2)}{N}} = \sqrt{\frac{15942}{8}} = \sqrt{1992} = 44.6$$

$$1.5\,\sigma = 44.6 \times 1.5 = 67$$

The counts 575 and 425 deviate from the mean by more than 67, therefore the procedure should be repeated.

Urobilinogen Excretion

Measurement of the breakdown products of hemoglobin in the recipient of a blood transfusion should give some evidence as to whether the donor's cells are disintegrating in the circulation with great rapidity. The quantitative determination of fecal urobilinogen will provide significant data when the rate of breakdown of hemoglobin is relatively fast. Smaller departures from normal can be detected only by the agglutination technique.

As employed by various workers[32,33] the procedure consists in the quantitative estimation of the total fecal excretion of urobilinogen in the recipient on the day of transfusion and for about seven days thereafter. If the rate of destruction of hemoglobin is greatly in excess of normal, significant increases in the excretion of pigment is found in the feces.

DETERMINATION OF UROBILINOGEN IN FECES (Watson[34])

Equipment and Materials. Waxed paper cartons of approximately 1000 ml. capacity. Large mortar and pestle. Small evaporating dish. Graduate cylinder of 100 ml. capacity. Erlenmeyer flask of 1000 ml. capacity. Graduated pipettes of 10 ml. capacity. Small separatory funnel. Chemical test tubes. Filter paper. Small glass funnel. Hellige type colorimeter for phenolsulfonphthalein

with permanent glass standard. 20 per cent ferrous sulfate solution (20 gm. ground crystals of $FeSO_4 \cdot 7H_2O$ in 92 ml. distilled water). Sodium hydroxide solution (10 per cent NaOH). Modified Ehrlich's reagent (0.7 gm. pure p-dimethylaminobenzaldehyde, 150 ml. concentrated hydrochloric acid, and 100 ml. distilled water). Saturated aqueous solution of sodium acetate. Petroleum ether (purified by standing over concentrated sulfuric acid for several days, then repeated washing with distilled water, finally distillation). Glacial acetic acid.

Procedure

1. *Preparation of Fecal Sample.* Collect the entire fecal output for four days in an opaque wax carton (during the period of collection the container should be kept in a cool place). Weigh the entire amount of feces for the four day period. If the consistency is soft, thoroughly mix the entire specimen in the container; if dry, mix in a large mortar with a pestle; if partly liquid and partly solid, pour off the liquid and make determinations on the two portions separately. Weigh out 10 gm. of the mixed feces on filter paper or in a small evaporating dish, depending on the amount of liquid present. Grind the aliquot into a paste in the mortar with from 10 to 20 ml. of a 90 ml. amount of distilled water, finally adding the remainder of the water and mixing thoroughly. Pour the supernatant fine suspension into a 1000 ml. Erlenmeyer flask which contains 100 ml. of 20 per cent ferrous sulfate solution. Grind the remaining material in the mortar with small amounts of distilled water and pour into the flask until a total of about 200 ml. has been added, washing the mortar clean. Finally add to the flask 100 ml. of 10 per cent NaOH, cork, shake, and set aside in the dark for one hour.

2. *Trial of Color Intensity.* Filter a small amount of the material from the flask and place 2 to 3 ml. of the filtrate in a test tube. Add an equal amount of Ehrlich's reagent. To the mixture add 3 to 4 ml. of saturated aqueous solution of sodium acetate and note the intensity of the color which develops. The color should indicate the volume of filtrate to be employed in step 3: very intense color, 1 ml. or less; moderately intense, 2 ml.; pale red, 3 to 5 ml.; faint, 10 to 30 ml.; color absent, 50 ml.

3. *Actual Determination.* Place the volume of filtrate indicated from step 2 in a separatory funnel and dilute to 20 ml. (if less than that volume was selected). Cover the filtrate with 20 to 30 ml. of pure petroleum ether and strongly acidify with glacial acetic acid. Shake the mixture vigorously for several seconds. After the layers have separated, extract the aqueous solution twice more with petroleum ether and discard the watery solution. Wash the petroleum ether solution twice in distilled water. Add 1 to 2 ml. of Ehrlich's solution to the petroleum ether extract and shake vigorously to extract the urobilinogen. Add at least twice the volume of saturated

sodium acetate solution, which results in the development of color. Shake this mixture vigorously. Withdraw the aqueous solution from the separatory funnel and save. Again shake the petroleum ether with a small amount of Ehrlich's reagent followed by sodium acetate solution. If more than a faint color develops, save the aqueous solution and again extract the petroleum ether until no color results.

4. *Colorimetric Reading.* Carry this out without delay. Make up the colored solution to an amount convenient for calculation. Place a portion in the right hand tube of the Hellige colorimeter. If the color is found to be more than 50 per cent of the standard, dilute the solution still further so that comparison may be made in the range of 20 to 50 per cent of the standard.

Calculation.

$$\frac{\text{Ml. Fe(OH)}_2\text{ mixture}}{\text{Gm. fecal sample}} \times \frac{\text{ml. final colored solution}}{\text{ml. filtrate used}} \times \%\text{ of color standard} \times \frac{\text{total gm. feces}}{100} \times \frac{1}{\text{number of days of collection}} = \text{mg.}$$

urobilinogen per day.

Examples.

Normal feces: $\frac{500}{10} \times \frac{45}{3} \times 0.35 \times 2.8 \times \frac{1}{4} = 183.7$ mg.

Acholic feces: $\frac{150}{10} \times \frac{10}{40} \times 0.5 \times 4.2 \times \frac{1}{4} = 1.9$ mg.

Radioactive Iron Method

Radioactive isotopes of iron may be employed to "tag" the donor's erythrocytes in the circulation of the recipient after a transfusion. The two isotopes suitable for this purpose are Fe^{59}, with a half life of forty-seven days, and Fe^{55}, having a half life of five years. The iron is incorporated in the salts ferrous sulfate, ferrous ammonium sulfate, or ferric ammonium sulfate. Donors are given suitable doses of the salts orally, intramuscularly, or intravenously. In the course of several weeks the tagged iron has been synthesized into the hemoglobin molecule of the erythrocytes, so that the cells are radioactive. A small volume of the donor's blood is injected into the recipient intravenously and the amount of radioactivity of the recipient's blood is indicative of the quantity of intact donor's cells in the circulation. The method is sufficiently sensitive that 0.0005 ml. of transfused erythrocytes may be measured in 1 ml. of the recipient's blood with an accuracy of ± 10 per cent.

This is the most precise measurement available of the donor's cells in the recipient's circulation. However, there are severe limitations which make the procedure impractical for some problems. The methods are highly technical, requiring expensive apparatus and workers skilled in handling radioactive substances. The radioactivity of the recipient's blood ceases to be a reliable index of the amount of donor's cells within forty-eight hours after transfusion, because the destroyed cells are metabolized and the isotope is utilized by the recipient in making new erythrocytes. The procedure is of greatest value in determining the number of donor's cells destroyed during the first few hours after transfusion, whereas the agglutination technique must be employed if the rate of loss of cells is to be studied after forty-eight hours.

The procedures are too complicated to be described in this work and the reader is referred to special papers on the subject for details.[35, 36]

COPPER SULFATE METHOD FOR MEASURING SPECIFIC GRAVITY

During World War II a method[37] of measuring hemoglobin and plasma protein concentrations was devised utilizing the determination of the specific gravity of the proteins. The procedure is so simple and accurate that it should find a wide use in civilian hospitals, particularly in problems associated with blood transfusion.

When a drop of protein solution is immersed in a solution of copper sulfate, a sac of copper proteinate forms on the surface of the drop and prevents a change in its contents for twenty to thirty seconds. If the specific gravity of the protein exceeds that of the copper sulfate solution, the drop sinks; when the protein is lighter than the copper sulfate, the drop tends to rise in the solution. By employing copper sulfate solutions of known graded densities the specific gravity of protein solutions may be determined with great accuracy.

The hemoglobin concentration of whole blood may be determined from a nomogram when the densities of whole blood and plasma have been estimated by this procedure. The hematocrit may be derived directly from the hemoglobin concentration if the assumption is made that the mean corpuscular hemoglobin is normal.

The advantages of the procedure are: (1) The measurements are sufficiently accurate for any clinical application. (2) The manipulations and interpretations are extremely simple. (3) A stable base is not required, e.g., the tests may be performed on shipboard. (4) The apparatus and materials are readily obtainable

and economical. (5) No temperature correction is needed because the temperature coefficient of the copper sulfate solutions is approximately equal to that of blood and plasma. (6) No constancy in the size of the drops is demanded so that calibrated pipettes are not necessary in performing the tests.

Preparation of Copper Sulfate Solutions

MAKING STOCK SOLUTION BY WEIGHING

This is the simpler procedure if pure copper sulfate ($CuSO_4 . 5H_2O$) can be obtained. The compound should be purchased as "fine crystals," otherwise it should be pulverized in a mortar before using. It may be tested for the theoretical five molecules of water of crystallization by drying 2 or 3 gm. in Pyrex weighing bottles to constant weight at 300° to 350° C. for two or three hours. It should lose 36.06 per cent of its weight in the conversion to the anhydrous salt. The observed loss of weight should be within 0.02 per cent of this value. The "analytic reagent" grade usually answers these specifications. The crystals should be kept in a tightly stoppered bottle until used.

Equipment and Materials. A balance weighing up to 200 gm. with an accuracy of 0.1 gm. A stoppered bottle of 4 liter capacity. Volumetric flask of 1000 ml. capacity. A pipette of 10 ml. capacity, graduated in 0.1 ml. A funnel 8 inches (20 cm.) in diameter. Centigrade thermometer for liquids at room temperature. Pure $CuSO_4 . 5H_2O$ in "fine crystals" or pulverized.

Procedure. Weigh out 170 gm. pure $CuSO_4 . 5H_2O$ and place in the 4 liter bottle. Fill the 1000 ml. volumetric flask to the mark with distilled water. Read the temperature of the water and consult table XIV for the additional volume of water to be added.

TABLE XIV

Volume of Water to be Added to 170 Grams $CuSO_4 5H_2O$ to Make a Stock Solution of Specific Gravity 1 1000

Temperature of water °C	Ml water to 170 gm. $CuSO_4$	Temperature of water °C	Ml. water to 170 gm. $CuSO_4$
10	1003 6	26	1006.5
12	1003 8	28	1007 0
14	1004.0	30	1007.7
16	1004 3	32	1008 3
18	1004 7	34	1008.9
20	1005 1	36	1009.6
22	1005.5	38	1010.4
24	1006 0	40	1011.2

Add this amount to the flask with a graduated pipette. Empty the flask and carefully drain the contents into the 4 liter bottle, stopper, and mix until all the crystals are dissolved. Make up two more liters in a similar manner and add to the 4 liter bottle. Label *Stock Solution, $CuSO_4$, Sp.Gr. 1.1000.*

MAKING STOCK SOLUTION FROM SATURATED $CuSO_4$

This method has the advantage of not requiring accurate weighing of the copper sulfate crystals. Furthermore, variation from the theoretical five molecules of water of crystallization in the salt will not preclude attaining the desired concentration. The method is more difficult in that it demands precise adherence to the directions.

Equipment and Materials. Three stoppered bottles, each with a capacity of about 4 liters. Approximately 4 pounds (2 kg.) of "fine crystals" or pulverized copper sulfate, $CuSO_4.5H_2O$ (the particles should be of sufficient fineness to pass a 20 mesh sieve). Centigrade thermometer for fluids at room temperature. A funnel approximately 8 inches (20 cm.) in diameter. Coarse filter paper or cotton for filtering. Graduate cylinder of 500 ml. capacity. Volumetric flask of 1000 ml. capacity. Watch or clock showing minutes. About 5 liters of distilled water at room temperature.

Procedure. Place the funnel and filter paper or cotton in a 4 liter bottle. Put about 4 pounds (2 kg.) of finely crystallized or pulverized copper sulfate in a second 4 liter bottle and add about 2.5 liters of distilled water. Stopper the bottle and shake vigorously, with frequent inversions of the bottle, *for exactly five minutes*. This may be accomplished by placing the bottle in a shaking machine or having two persons alternate manual agitation every minute or so. Measure the temperature to the nearest 0.5° C. *immediately after shaking has ceased.* This is considered the instant at which saturation has been achieved and the temperature precisely at this time must be obtained to carry out the procedure. As soon as the temperature has been measured, quickly decant off the fluid from the undissolved crystals and pour into the first 4 liter bottle through the filter. From Table XV ascertain the volume of saturated solution required at the temperature observed to be diluted to 1000 ml. Measure this amount in a 500 ml. cylinder and pour it into the 1000 ml. volumetric flask, letting the solution drain from the cylinder for thirty seconds. Fill the volumetric flash with distilled water up to the mark and mix the contents by inversion. There will be some contraction of the volume from the mixing, so permit the solution to stand in the flask for a minute or so and then add distilled water to

TABLE XV

Volume of Saturated $CuSO_4$ Solution, as Determined by Its Temperature at the Time of Saturation, to be Diluted to 1000 ml. to Give a Stock Solution of Specific Gravity of 1.1000

°C	Ml.	°C	Ml.	°C	Ml.	°C	Ml.
10.0	587	17.5	509	25.0	453	32.5	410
10.5	581	18.0	505	25.5	450	33.0	407
11.0	575	18.5	501	26.0	446	33.5	404
11.5	569	19 0	497	26.5	443	34.0	401
12.0	563	19.5	493	27.0	440	34.5	398
12.5	557	20 0	489	27.5	438	35.0	395
13.0	552	20.5	485	28.0	435	35.5	392
13.5	546	21.0	481	28.5	432	36.0	389
14.0	541	21.5	477	29.0	429	36.5	387
14.5	536	22.0	474	29.5	427	37.0	384
15.0	531	22.5	470	30 0	424	37.5	381
15 5	527	23 0	466	30.5	421	38.0	378
16 0	522	23.5	463	31.0	418	38 5	374
16.5	518	24 0	459	31.5	415	39.0	371
17.0	514	24.5	456	32.0	412	39.5	368
						40.0	365

the mark. After mixing, empty the flask into the 4 liter bottle. Prepare three more liters of stock solution with the same cylinder and volumetric flask and add the amounts to the same 4 liter bottle. Label the bottle *Stock Solution, $CuSO_4$, Sp.Gr. 1.1000.*

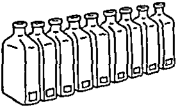
If the temperatures of the saturated solution, the stock solution, and the standard solutions are not within 5° C. of each other when their volumes are measured, there will be appreciable errors in the densities of the standard solutions. It is desirable, therefore, that all equipment and materials, including the distilled water employed for the diluent and solvent, should be permitted to attain room temperature before the measurement of volumes is begun.

PREPARATION OF STANDARD SOLUTIONS OF COPPER SULFATE

If the entire range of specific gravities pertaining to blood and plasma is to be covered, it is recommended that sixty standards be made in the range from 1.016 to 1.075 in intervals of 0.001. Seven more standards from 1.008 to 1.015 will include the range for ascitic fluid. If only approximate densities are to be measured, sixteen standards are sufficient with a range from 1.016 to 1.076 at intervals of 0.004. Directions will be given for making up standards in 100 ml. quantities; if smaller amounts are required, the values may be divided accordingly.

Equipment and Materials. Eighteen, sixty-two, or sixty-nine prescription bottles with plastic screw caps and a capacity of about 4 ounces (120 ml.). One burette measuring a total of 100 ml. graduated in intervals of 0.1 ml. One or more volumetric flasks of 100 ml. capacity. One Erlenmeyer flask of 250 to 500 ml. capacity. Stock $CuSO_4$ solution of specific gravity 1.1000.

Procedure. Label the prescription bottles with the specific gravities of the standards which they are to contain. It is suggested that the labels be placed at the bottoms of the narrow sides of the bottles



so that the flat sides of the bottles may be approximated, thus a minimum of table space may be occupied by the line of bottles.

TABLE XVI

Milliliters of Stock Copper Sulfate Solution of Gravity 1 1000 to be Diluted to 100 Ml. to Prepare Standard Solutions with Stated Specific Gravity ±0 0001

Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml of Stock Solution	Specific Gravity	Ml of Stock Solution
1.008	7.33	1 026	25 12	1 046	45 0	1 066	65 0
9	8 32	27	26.10	47	46 0	67	66 0
10	9 31	28	27 08	48	47 0	68	67 0
		29	28.06	49	48.0	69	68 1
		30	29 04	50	49 0	70	69 1
1 011	10 30	1 031	30.0	1 051	50 0	1 071	70 2
12	11.29	32	31 0	52	51 0	72	71 2
13	12.28	33	32 0	53	52 0	73	72 2
14	13 27	34	33.0	54	53 0	74	73 3
15	14 26	35	34 0	55	54 0	75	74 3
1 016	15 25	1.036	35 0	1.056	55 0		
17	16 24	37	36 0	57	56 0		
18	17 23	38	37.0	58	57 0		
19	18 22	39	38 0	59	58 0		
20	19.21	40	39 0	60	59 0		
1.021	20.20	1 041	40 0	1 061	60 0		
22	21.19	42	41.0	62	61 0		
23	22 17	43	42 0	63	62 0		
24	23.15	44	43 0	64	63.0		
25	24 94	45	44.0	65	64 0		

TABLE XV

Volume of Saturated $CuSO_4$ Solution, as Determined by Its Temperature at the Time of Saturation, to be Diluted to 1000 ml. to Give a Stock Solution of Specific Gravity of 1.1000

°C	Ml	°C	Ml.	°C	Ml.	°C	Ml.
10.0	587	17.5	509	25.0	453	32.5	410
10.5	581	18.0	505	25.5	450	33.0	407
11.0	575	18.5	501	26.0	446	33.5	404
11.5	569	19.0	497	26.5	443	34.0	401
12.0	563	19.5	493	27.0	440	34.5	398
12.5	557	20.0	489	27.5	438	35.0	395
13.0	552	20.5	485	28.0	435	35.5	392
13.5	546	21.0	481	28.5	432	36.0	389
14.0	541	21.5	477	29.0	429	36.5	387
14.5	536	22.0	474	29.5	427	37.0	384
15.0	531	22.5	470	30.0	424	37.5	381
15.5	527	23.0	466	30.5	421	38.0	378
16.0	522	23.5	463	31.0	418	38.5	374
16.5	518	24.0	459	31.5	415	39.0	371
17.0	514	24.5	456	32.0	412	39.5	368
						40.0	365

the mark. After mixing, empty the flask into the 4 liter bottle. Prepare three more liters of stock solution with the same cylinder and volumetric flask and add the amounts to the same 4 liter bottle. Label the bottle *Stock Solution, $CuSO_4$, Sp.Gr. 1.1000.*

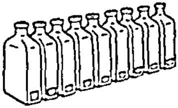
If the temperatures of the saturated solution, the stock solution, and the standard solutions are not within 5° C. of each other when their volumes are measured, there will be appreciable errors in the densities of the standard solutions. It is desirable, therefore, that all equipment and materials, including the distilled water employed for the diluent and solvent, should be permitted to attain room temperature before the measurement of volumes is begun.

Preparation of Standard Solutions of Copper Sulfate

If the entire range of specific gravities pertaining to blood and plasma is to be covered, it is recommended that sixty standards be made in the range from 1.016 to 1.075 in intervals of 0.001. Seven more standards from 1.008 to 1.015 will include the range for ascitic fluid. If only approximate densities are to be measured, sixteen standards are sufficient with a range from 1.016 to 1.076 at intervals of 0.004. Directions will be given for making up standards in 100 ml. quantities; if smaller amounts are required, the values may be divided accordingly.

Equipment and Materials. Eighteen, sixty-two, or sixty-nine prescription bottles with plastic screw caps and a capacity of about 4 ounces (120 ml). One burette measuring a total of 100 ml. graduated in intervals of 0.1 ml. One or more volumetric flasks of 100 ml. capacity. One Erlenmeyer flask of 250 to 500 ml. capacity. Stock $CuSO_4$ solution of specific gravity 1.1000.

Procedure. Label the prescription bottles with the specific gravities of the standards which they are to contain. It is suggested that the labels be placed at the bottoms of the narrow sides of the bottles



so that the flat sides of the bottles may be approximated, thus a minimum of table space may be occupied by the line of bottles.

TABLE XVI

Milliliters of Stock Copper Sulfate Solution of Gravity 1.1000 to be Diluted to 100 Ml. to Prepare Standard Solutions with Stated Specific Gravity ±0.0001

Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml. of Stock Solution
1.008	7.33	1.026	25.12	1.046	45.0	1.066	65.0
9	8.32	27	26.10	47	46.0	67	66.0
10	9.31	28	27.08	48	47.0	68	67.0
		29	28.06	49	48.0	69	68.1
		30	29.04	50	49.0	70	69.1
1.011	10.30	1.031	30.0	1.051	50.0	1.071	70.2
12	11.29	32	31.0	52	51.0	72	71.2
13	12.28	33	32.0	53	52.0	73	72.2
14	13.27	34	33.0	54	53.0	74	73.3
15	14.26	35	34.0	55	54.0	75	74.3
1.016	15.25	1.036	35.0	1.056	55.0		
17	16.24	37	36.0	57	56.0		
18	17.23	38	37.0	58	57.0		
19	18.22	39	38.0	59	58.0		
20	19.21	40	39.0	60	59.0		
1.021	20.20	1.041	40.0	1.061	60.0		
22	21.19	42	41.0	62	61.0		
23	22.17	43	42.0	63	62.0		
24	23.15	44	43.0	64	63.0		
25	24.94	45	44.0	65	64.0		

TABLE XV

Volume of Saturated $CuSO_4$ Solution, as Determined by Its Temperature at the Time of Saturation, to be Diluted to 1000 ml. to Give a Stock Solution of Specific Gravity of 1.1000

° C	Ml.	° C	Ml.	° C	Ml.	° C	Ml.
10.0	587	17.5	509	25.0	453	32.5	410
10.5	581	18.0	505	25.5	450	33.0	407
11.0	575	18.5	501	26.0	446	33.5	404
11.5	569	19 0	497	26.5	443	34.0	401
12.0	563	19.5	493	27.0	440	34.5	398
12.5	557	20.0	489	27.5	438	35.0	395
13.0	552	20.5	485	28 0	435	35.5	392
13.5	546	21.0	481	28.5	432	36.0	389
14.0	541	21 5	477	29.0	429	36.5	387
14.5	536	22.0	474	29.5	427	37.0	384
15.0	531	22 5	470	30.0	424	37.5	381
15.5	527	23.0	466	30.5	421	38.0	378
16 0	522	23.5	463	31 0	418	38 5	374
16.5	518	24 0	459	31.5	415	39.0	371
17.0	514	24.5	456	32.0	412	39 5	368
						40.0	365

the mark. After mixing, empty the flask into the 4 liter bottle. Prepare three more liters of stock solution with the same cylinder and volumetric flask and add the amounts to the same 4 liter bottle. Label the bottle *Stock Solution, $CuSO_4$, Sp.Gr. 1.1000.*

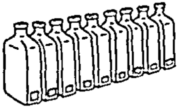
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Equipment and Materials. Eighteen, sixty-two, or sixty-nine prescription bottles with plastic screw caps and a capacity of about 4 ounces (120 ml.). One burette measuring a total of 100 ml. graduated in intervals of 0.1 ml. One or more volumetric flasks of 100 ml. capacity. One Erlenmeyer flask of 250 to 500 ml. capacity. Stock $CuSO_4$ solution of specific gravity 1.1000.

Procedure. Label the prescription bottles with the specific gravities of the standards which they are to contain. It is suggested that the labels be placed at the bottoms of the narrow sides of the bottles



so that the flat sides of the bottles may be approximated, thus a minimum of table space may be occupied by the line of bottles.

TABLE XVI

Milliliters of Stock Copper Sulfate Solution of Gravity 1.1000 to be Diluted to 100 Ml. to Prepare Standard Solutions with Stated Specific Gravity ±0.0001

Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml. of Stock Solution	Specific Gravity	Ml. of Stock Solution
1.008	7.33	1.026	25.12	1.046	45.0	1.066	65.0
9	8.32	27	26.10	47	46.0	67	66.0
10	9.31	28	27.08	48	47.0	68	67.0
		29	28.06	49	48.0	69	68.1
		30	29.04	50	49.0	70	69.1
1.011	10.30	1.031	30.0	1.051	50.0	1.071	70.2
12	11.29	32	31.0	52	51.0	72	71.2
13	12.28	33	32.0	53	52.0	73	72.2
14	13.27	34	33.0	54	53.0	74	73.3
15	14.26	35	34.0	55	54.0	75	74.3
1.016	15.25	1.036	35.0	1.056	55.0		
17	16.24	37	36.0	57	56.0		
18	17.23	38	37.0	58	57.0		
19	18.22	39	38.0	59	58.0		
20	19.21	40	39.0	60	59.0		
1.021	20.20	1.041	40.0	1.061	60.0		
22	21.19	42	41.0	62	61.0		
23	22.17	43	42.0	63	62.0		
24	23.15	44	43.0	64	63.0		
25	24.94	45	44.0	65	64.0		

Fill the Erlenmeyer flask with stock copper sulfate solution. From this, fill the burette as needed. Consult Table XVI and measure the proper volume of stock solution into a 100 ml. volumetric flask. Fill the flask to the mark with distilled water. Mix the contents and pour into the proper prescription bottle. Rinse the volumetric flask once with distilled water, refill the burette with stock solution, and prepare the next standard.

In addition to the series of standards desired, make up an extra standard for each of the specific gravities 1.028 and 1.060 in the same sized bottles and label *Control Standards*. Add one-fortieth its volume of normal whole blood to each of the two control standards. *Keep these for comparison with the used standards*. When blood is added to the copper sulfate solutions the protein is precipitated and finally sinks to the bottom. The used standards should be discarded when the mass of precipitated protein approximates in amount that in the control standards. One hundred milliliters of a copper sulfate solution will accommodate approximately 100 small drops of blood or plasma before the accumulated protein precipitate alters the specific gravity of the solution enough to introduce a significant error into the determinations.

Gravity Analysis of Blood and Plasma

Collection of Blood Samples. If venous blood is to be analyzed, *the tourniquet should not be applied to the arm for over a minute*, so that hemoconcentration from forcing fluid into the tissues is prevented. Capillary blood from skin puncture should not be employed to determine the hemoglobin concentration in cases of shock because it may give a value which is relatively too high in comparison with that from blood in the larger vessels.

Anticoagulants. Whole blood without an anticoagulant may be expelled from the syringe into the standard solutions if the maneuver can be performed before clotting occurs. The anticoagulants which may be employed with copper sulfate solutions are either heparin or a mixture of potassium and ammonium oxalate. Sodium citrate must *not* be used. Heparin is added in the proportion of 0.2 mg. per 1 ml. of blood. Tubes containing oxalate are prepared as follows: Dissolve 3 gm. ammonium oxalate and 2 gm. potassium oxalate in 250 ml. of water to make a 2 per cent solution. Mark serologic test tubes at a level indicating 5 ml. Add to each tube 0.25 ml. of the 2 per cent oxalate solution, spread in a thin film in the lower one half of the tube. Place the tubes in an incubator with the temperature below 50° C. or in a vacuum desiccator, until the solution has dried.

Serum may be used instead of plasma to determine the density of plasma proteins, but it should be recalled that the specific gravity will be less than that of plasma from which it is derived because of the loss of fibrinogen which is present in plasma in a concentration of more than 0.2 gm. per 100 ml.

MEASUREMENT OF DENSITY OF BLOOD FRACTIONS

Equipment and Materials. Series of copper sulfate standards. Bottles containing oxalate mixture or heparin for blood samples. Capillary pipettes. Syringes, needles, tourniquet, sponges, and alcohol.

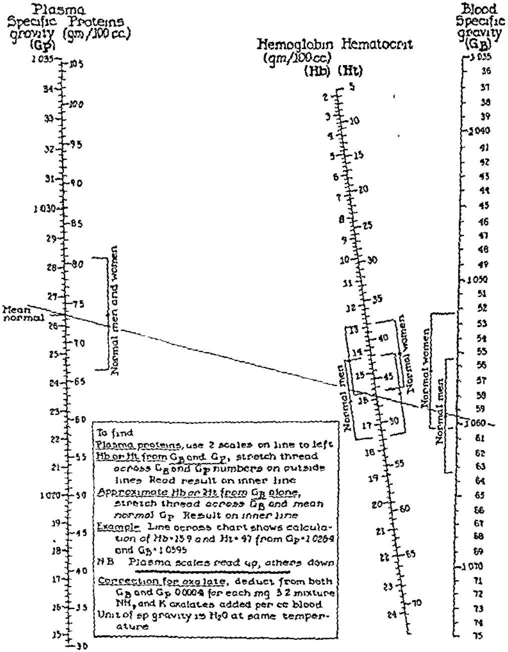
Procedure. Deliver a drop of whole blood from the needle of a syringe, or dispense a drop of oxalated or heparinized blood or plasma from a capillary pipette. Permit the drop to fall into the standard copper sulfate solution from a distance of about 1 cm. above. Observe the behavior of the drop in the copper sulfate. It will sink beneath the surface from the momentum of its fall. Within five seconds it will rise slightly if it is lighter than the solution, fall slowly if heavier, or remain suspended if its density is equal to that of the copper sulfate. Twenty to thirty seconds after immersion the drop will disintegrate and the precipitated protein will fall to the bottom of the bottle. The size of the drop of blood has no bearing on the results of the test but, if the drops are small, more tests can be made with a copper sulfate standard before a new standard must be employed. The size of the drop may be reduced not only by using a smaller orifice in the pipette but also by greasing the tip with vaseline mixed with a little caprylic alcohol.



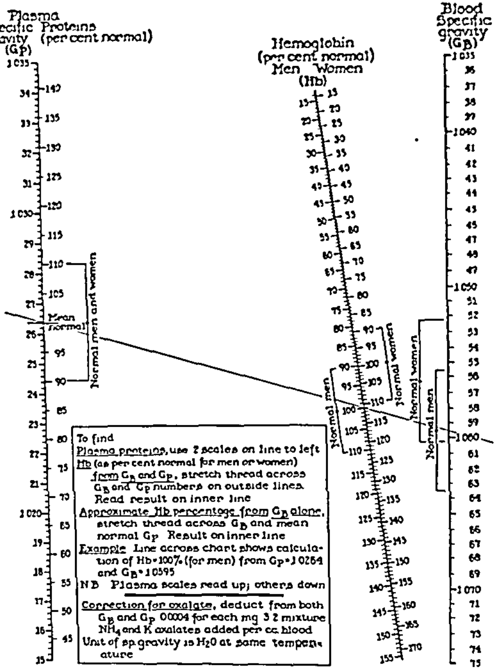
Test the blood or plasma in a series of copper sulfate standards until one is found in which the drop remains suspended from five to ten seconds, or the point in the series is located at which the drop is lighter than one standard but heavier than the next.

Calculations. *Plasma Protein.* With the exception of severe renal disease in which the plasma lipids are greatly increased, the following determination is accurate within 0.4 gm. per 100 ml.:

$$\text{Gm. protein per 100 ml. plasma} = 370 \times (\text{plasma density} - 1.0070)$$



Line chart for calculating concentrations of plasma proteins, hemoglobin and hematocrit from specific gravities of plasma and blood. (Phillips, Van Slyke, Dole, Emerson, Hamilton and Archibald in TM 8-227, *Methods for Laboratory Technicians*, War Department, October, 1946.)



Line chart for calculating percentages of normal plasma proteins and hemoglobin from specific gravities of plasma and blood (Phillips, Van Slyke, Dole, Emerson, Hamilton and Archibald in TM 8-227, Methods for Laboratory Technicians, War Department, October, 1946)

Hemoglobin Concentration. For normal blood or that in which the mean corpuscular hemoglobin concentration is normal, the hemoglobin values by the following formula are accurate within ±0.3 gm. per 100 ml.:

Gm. hemoglobin per 100 ml. blood

$$= 33.9 \times \frac{\text{blood density} - \text{plasma density}}{1.0970 - \text{plasma density}}$$

When there is great deviation from normal in mean corpuscular hemoglobin concentration the error is not greater than ±0.6 gm. per 100 ml.

Hematocrit. This calculation assumes a normal concentration ot hemoglobin in the erythrocytes. When this assumption is correct, the error of the method is ±2 ml. per 100 ml. of cells.

$$\text{Ml. cells in 100 ml. blood} = 100 \times \frac{\text{blood density} - \text{plasma density}}{1.0970 - \text{plasma density}}$$

Oxygen Capacity. This calculation is based on the estimation of the hemoglobin concentration and is subject to same sources of error.

Ml. oxygen bound by 100 ml. blood

$$= 46.1 \times \frac{\text{blood density} - \text{plasma density}}{1.0970 - \text{plasma density}}$$

TESTING OF BLOOD DONORS FOR MINIMUM HEMOGLOBIN

During World War II the American Red Cross Blood Donor Service was confronted with the problem of developing a simple, rapid, accurate method of determination of hemoglobin concentration for the acceptance or rejection of blood donors. An adaptation of the copper sulfate method was devised by Dr. William Thalhimer. It is based on the principle of determining whether a drop of the prospective donor's blood has a density greater or less than that of a single copper sulfate standard. The density of the standard depends upon the minimum concentration of hemoglobin which is considered desirable for a blood donor. The standard chosen for the Red Cross project had a specific gravity of 1.052. If the density of the plasma approximates the mean normal, the standard has a specific gravity equivalent to 12.2 gm. of hemoglobin per 100 ml. of blood.

The test, then, rejects prospective donors with a hemoglobin concentration of less than 12.2 gm. per 100 ml. and accepts those with a higher concentration. Should the density of the plasma be less than normal but the hemoglobin concentration be approximately the minimum acceptable, the test would reject the candidate, but this error would be in the directio[illegible]

Equipment and Materials. Copper sulfate solution of density 1.052 (or any other density which may be chosen). Glass capillary tubes, approximately 80 mm. long and 1 mm. inside bore (used for smallpox vaccine). Rubber bulbs with holes at both ends, to fit capillary tubes. Lancet, sponges, and alcohol.

Procedure

1. *Preparation.* Wash the finger of the donor with alcohol and permit it to *dry completely*. While the finger is drying, place a rubber bulb on one end of a glass capillary tube. Make a clean puncture of the skin with a sterile lancet so that the blood flows freely.

2. *Collection of Blood.* Grasp the bulb on the capillary with the thumb and middle finger, leaving the hole of the bulb open. Hold the tube horizontally so that the blood runs into the free end until about three quarters of the tube is filled.

3. *Test.* Hold the tube vertically with the tip about 1 cm. above the surface of the copper sulfate solution. Cover the hole in the bulb with the index finger and gently compress the sides of the bulb with the thumb and middle finger so that a drop of blood is expelled.

4. *Observation.* After the drop has lost momentum, note whether it rises slightly, remains suspended for twenty seconds, or falls promptly.

Interpretation. If the drop rises, the hemoglobin concentration is less than the acceptable minimum and the donor is rejected. The hemoglobin value is greater than the acceptable minimum if the drop sinks promptly.

MEASUREMENT OF BLOOD VOLUME

Occasionally it is desirable to measure the circulating blood volume of a patient who has hemorrhagic shock or some other disorder in which there is depletion of blood or plasma. Several procedures are available for this determination.

Change in Blood Density After Plasma Infusion[33]

This method is based upon the determination of the change in the specific gravity of the blood of the subject produced by the rapid infusion of a measured volume of plasma. The densities of the whole blood and the plasma solution are measured by the copper sulfate method.

Equipment and Materials. One series of copper sulfate standards of known graded specific gravities. Three dry syringes (1 ml. capacity or greater) fitted with 20 gauge needles, 1½ inches (3.75 cm.) in length. A steel tape measure, 2 meters long, marked in

centimeters. One volume of isotonic plasma, measured accurately in liters (preferably between 0.5 and 1.5 liters) in an apparatus suitable for giving an infusion. Sponges, alcohol and tourniquet. Optional: one log-log slide rule.

Procedure

1. *First Blood Sample.* Place the copper sulfate standards near the patient. Withdraw from the vein of the patient approximately 1 ml. of blood and determine its density by expressing drops from the syringe directly into the copper sulfate standards before clotting occurs (pp. 214 to 215). Designate the value of the density as G_{B1}.

2. *Plasma Infusion.* Measure the volume of the plasma to be infused in fractions of a liter. Designate this value as V_S. Take up 1 ml. of the plasma in a second syringe. Then start the infusion into the vein of the patient at a rate between 50 and 100 ml. per minute. While the infusion is being given, determine the specific gravity of the plasma in the copper sulfate solutions and designate this value as G_S.

3. *Second Blood Sample.* Ten minutes after the infusion has been stopped withdraw another sample of blood from the patient in the third syringe and determine its specific gravity as in step 1. Designate the value so obtained as G_{B2}.

4. *Measurement of the Patient.* Measure the height of the patient in centimeters with the steel tape. Designate this value as H. If the weight of the patient cannot be obtained by measurement or questioning, make an approximation by guess. Express the weight in kilograms (divide the number of pounds by 2.2) and designate this value as W.

Calculations. *Observed Blood Volume:* V_{B1} = blood volume of the patient in liters *before infusion.*

$$V_{B1} = V_S\left(\frac{G_{B2} - G_S}{G_{B1} - G_{B2}}\right)$$

Mean Normal Blood Volume: A = surface area of the patient in square meters.

$$A = (H^{0.725} \times W^{0.425})\ 0.007184$$

Mean normal blood volume in liters = 4.48A − 2.11

CHANGE IN HEMOGLOBIN CONCENTRATION AFTER PLASMA INFUSION

The authors[38] of the preceding method devised a modification in which the hemoglobin concentration is estimated before and after the infusion of a measured volume of plasma. This requires the use of oxalated blood samples and centrifugation of the samples to obtain the density of whole blood and plasma separately.

Equipment and Materials. One series of copper sulfate standards. Three dry syringes (1 ml. capacity or greater) fitted with 20

gauge needles, 1½ inches (3.75 cm.) in length. One steel tape, 2 meters in length, calibrated in centimeters. Sponges, alcohol, and tourniquet. One volume of plasma, measured accurately in liters (preferably between 0.5 and 1.5 liters) in an apparatus suitable for giving an infusion. Two tubes containing oxalate mixture (p. 214). A centrifuge. Drop pipettes.

Procedure

1. *First Blood Sample* Collect approximately 1 ml of blood from the vein of the patient, place in a tube containing oxalate mixture, and thoroughly mix. Label the tube B_1

2. *Plasma Infusion* Measure the volume of the plasma to be infused in fractions of a liter Designate this value as V_S. Take up a 1 ml sample of the plasma in a syringe and Label S Inject the infusion into the vein of the patient at a rate of 50 to 100 ml per minute.

3. *Second Blood Sample.* Ten minutes after the infusion of plasma is completed collect about 1 ml. of blood from the vein of the patient in the third syringe and place in an oxalated tube Label this B_2.

4. *Measurement of the Patient.* Measure the height of the patient in centimeters. Designate this value as H. If the weight of the patient cannot be measured, obtain an approximation from questioning or guessing. Express the weight in kilograms (divide the number of pounds by 2.2) and designate this value as W.

5. *Densities of the Samples.* With the standard copper sulfate solutions determine the specific gravities of whole blood from B_1 and B_2 and designate these values as G_{B1} and G_{B2} respectively. Centrifuge the remainder of the whole blood samples and determine the densities of the plasma from each, designating the values G_{P1} and G_{P2}. Also determine the density of the plasma S and designate the value as G_S

Calculations. *Hemoglobin Concentration:* Hb = hemoglobin in grams per 100 ml. of blood.

$$Hb_1 = 33.9 \times \frac{G_{B1} - G_{P1}}{1.097 \times G_{P1}} \text{ and } Hb_2 = 33.9 \times \frac{G_{B2} - G_{P2}}{1.097 \times G_{P2}}$$

Observed Blood Volume: V_{B1} = blood volume in liters *before infusion*

$$V_{B1} = V_S \left(\frac{Hb_2}{Hb_1 - Hb_2} \right)$$

Mean Normal Blood Volume: A = surface area of the patient in square meters

$$A = (H^{0.725} \times W^{0.425})\ 0.007184$$

Mean normal blood volume in liters = 4.48A − 2.11

Accuracy of the Infusion Methods. The authors of the procedures state that the blood volume may be estimated with an accuracy of ±15 per cent.

EVANS BLUE DYE (T-1824) METHOD FOR BLOOD VOLUME (modified from Gregersen[39])

This procedure depends upon the injection into the circulation of a carefully measured amount of the nontoxic Evans blue dye (T-1824) and the estimation of the concentration of the dye in the plasma after a suitable time has elapsed to permit thorough mixing. The dye forms a temporary combination with plasma albumin and therefore measures the plasma volume. The calculation of whole blood volume requires the hematocrit value and the plasma volume.

Gregersen's method employs the Nickerson Decade Photometer to obtain the optical density of the colored plasma. Undoubtedly this is an excellent instrument, but it has the disadvantage of being a single purpose apparatus. The method has been adapted here for the photoelectric colorimeter which is more likely to be available in the hospital laboratory.

Calibration of the Colorimeter. *Equipment and Materials.* A standard type of photoelectric colorimeter with cups of approximately 4 ml. capacity and color filter of 620 to 625 millimicrons (red). A 50 ml. volumetric flask. A serologic pipette of 1 ml. capacity, graduated in 0.01 ml. Several pipettes of 10 ml. capacity, graduated in 0.1 ml. A 5 ml. ampule of T-1824 of approximately 0.45 per cent concentration in water, suitable for intravenous injection. About 150 ml. of human plasma, diluted but slightly with anticoagulant. Twelve test tubes with at least 15 ml. capacity. Graph paper.

TABLE XVII

Volume of Dye	Volume of Plasma	Final Dilution of Dye	Equivalent Plasma Volume in Test
0.5 ml. undiluted dye	49.5 ml.	1/100	500 ml.
4.0 1/100 dilution	4.0	1/200	1000
3.0 " "	6.0	1/300	1500
2 0 " "	6.0	1/400	2000
2.0 " "	8.0	1/500	2500
1.0 " "	5.0	1/600	3000
1.0 " "	6 0	1/700	3500
1.0 " "	7.0	1/800	4000
1.0 " "	8.0	1/900	4500
1.0 " "	9.0	1/1000	5000
1.0 " "	10.0	1/1100	5500
1 0 " "	11.0	1/1200	6000
1.0 " "	12.0	1/1300	6500

Dilution of the Dye. Measure out accurately 0.5 ml. of the dye and expel this into the 50 ml. volumetric flask. Fill the flask to the mark with human blood plasma and mix thoroughly. This makes a 1/100 dilution of the dye. From the 1/100 dilution make the other dilutions according to Table XVII, using test tubes as containers.

Plotting the Curve. Place the 620 to 625 millimicron filter in the colorimeter. In one colorimeter cup put about 4 ml. of undyed plasma and, with the light of the colorimeter transmitted through the plasma, set the galvanometer dial at the Zero reading. Then place the dilutions of dye in other cups and read the amount of light transmission through them. The dials are read either in per cent of light transmission or in terms of optical density (2 − logarithm of per cent transmission). On the abscissae of the graph paper make a linear scale for equivalent plasma volumes in the test. On the ordinates make a scale for light transmission. If the values on the dial are given only in per cent light transmission, plot these on a logarithmic scale (using semi-log paper). If the values are obtained on the colorimeter dial in terms of optical density, plot these on a linear scale (using linear graph paper).

Equipment and Materials. Photoelectric colorimeter, properly calibrated for T-1824, with 620 to 625 millimicron filter and cups of approximately 4 ml. capacity. Ampules containing exactly 5 ml. of 0.45 per cent solution of T-1824, for intravenous injection. Three 10 ml. syringes and sharp 20 gauge needles, 1½ inches (3.75 cm.) in length. Two oxalated test tubes for 10 ml. of blood each. Sponges, alcohol, and tourniquet. Centrifuge. Series of copper sulfate standards. Drop pipettes. Sterile isotonic saline solution (0.9 per cent NaCl). Steel tape, 2 meters long, calibrated in centimeters.

Procedure

1. *Preparation of Dye.* Wash a syringe, with needle attached, with 2 to 3 ml. of sterile saline solution. Draw 5 ml from the contents of an ampule of dye into the syringe, without washing the sides of the ampule free from dye (the contents are adjusted to allow for this loss). Then carefully draw into the syringe 2 to 3 ml. of saline to wash the needle clean from the dye. Place the filled syringe within reach of the patient.

2. *First Blood Sample.* Insert the needle of a second syringe into the vein of the patient and withdraw about 10 ml. of blood without permitting air bubbles in the syringe. Leaving the needle in place, detach the filled syringe and pass it to an assistant who exchanges it for the syringe filled with dye. Attach the second syringe to the needle in the vein and inject the dye, withdrawing blood into the syringe and reinjecting once or twice to wash all the dye into the vein. In the meantime, have the assistant empty the syringe of blood by gently expelling it into a tube containing oxalate. Mix the blood gently with the anticoagulant and label the sample B_1.

3. *Second Blood Sample.* Exactly ten minutes after the injection of the dye, withdraw another 10 ml. blood sample with the third syringe and label it B_2.

4. *Densities of Whole Blood.* Determine the specific gravities of samples B_1 and B_2 in the copper sulfate standards and designate the values G_{B1} and G_{B2}. T ese values should be equal. Centrifuge the remainder of both samples until the supernatant plasma is clear. Transfer the plasma from B_1 to a colorimeter cup and designate it P_1 and the plasma of the second sample to another cup labeled P_2.

5. *Color Concentration.* Insert the filter in the colorimeter and adjust. Place the cup containing P_1 in the colorimeter and adjust the galvanometer dial to Zero when the light is transmitted through it. Then insert the cup with P_2, adjust the dial and record the reading.

6. *Densities of Plasma.* Determine the specific gravities of P_1 and P_2 in the copper sulfate standards and designate the values as G_{P1} and G_{P2} respectively. These values should be equal if there has been no loss of blood in the ten-minute period.

7. *Measurement of the Patient.* Measure the height (H) of the patient in centimeters with the steel tape. If the weight (W) of the patient cannot be measured, obtain the datum by questioning or by guessing.

Calculations. *Observed Plasma Volume.* Consult the calibration graph and find the equivalent plasma volume on the abscissa which corresponds to the point where the galvanometer reading obtained on P_2 intersects the calibration curve.

Observed Hematocrit. Solve the following equation:

$$\text{Ml. cells in 100 ml. blood} = 100 \times \frac{G_B - G_P}{1.0970 - G_P}$$

Observed Blood Volume. V_P = observed plasma volume; V_c = hemotocrit.

$$\text{Observed blood volume in ml.} = V_P + \left(\frac{V_c}{100} \times V_P\right)$$

Mean Normal Blood Volume. A = surface area of the patient in square meters.

$$A = (H^{0.725} \times W^{0.425})0.007184$$

Mean normal blood volume in liters = 4.48A − 2.11

Gibson and Evans[42] stated that there is as good a correlation between blood volume and body weight as there is with surface area or height. They published the following values for normal subjects:

Average values for plasma volume	Males, 43.08 ml. per kilogram
	Females, 41.5 ml per kilogram
Average values for blood volume	Males, 77 7 ml per kilogram
	Females, 66.1 ml. per kilogram

Radioactive Iron Method for Blood Volume[illegible]

Several techniques have been employed for the measurement of the circulating blood volume by labeling erythrocytes with radioactive isotopes of iron and transfusing them into the subject. The amount of radioactivity is determined and is taken as a measure of the circulating red cells. The hematocrit is determined and furnishes the other datum for calculating the whole blood volume.

The iron isotope Fe^{59} with a half life of forty-seven days has been most widely used but Fe^{55}, which has a half life of five years, has been employed by at least one group of investigators.[illegible] With either isotope the radioactive material is given orally or by intravenous injection to a suitable donor until his erythrocytes contain sufficient of the radioactive iron which has been synthesized into hemoglobin. A measured amount of his blood is transfused into the circulation of the subject and the concentration of radioactivity is measured by suitable techniques. The methods are too specialized to be given in this article.

Gibson *et al.* (*loc. cit.*) reported a difference in the whole blood volume of normal human subjects and dogs as determined by the radio iron technique and the Evans blue dye method. In human beings the volume as calculated by the radioactive iron method was related to the value obtained by the dye technique in an average ratio of 0.85. It will be noted that in the radioactive method the volume of circulating cells is actually measured and the plasma volume calculated from the hematocrit, whereas with the dye method the plasma volume is determined and the cells are estimated from the hematocrit. It is suggested that these discrepancies may be explained because there are differences between the hematocrit value of capillary blood and the blood in large vessels.

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CHAPTER 10

The Donor and the Collection of Blood

By Robert C. Hardin

Although the attention in blood transfusion is largely and properly directed to the recipient, the second individual who is intimately concerned is the donor. It is important that he be carefully selected that his blood not prove detrimental to the patient and that all possible precautions be taken to prevent him from suffering harm. The loss of 500 ml. of blood is well tolerated by the usual healthy individual. It cannot, however, be regarded as a normal physiologic experience. Although bleeding proceeds uneventfully in most instances, there are sometimes unfavorable responses in the donor which result in minor accidents or even in catastrophe.

CHOICE OF DONOR

Physical Qualifications. *Sex.* Persons of either sex may be used as donors. Females should not be bled during menstruation or pregnancy because they are in physiologic need of blood reserves at these times.

Age. The age limits for donors are admittedly arbitrary. Neither the adolescent nor the senile individual should be bled. The former is in a stage of rapid growth and can ill adjust to further strain. The latter is likely to be unable to compensate for even a minor degree of anemia. At both ends of the age scale there is considerable allowable variation which depends upon the actual physical state of the individual, so that the employment of a prospective donor must be left to the judgment of the transfusionist. When limits are employed with a certain amount of flexibility, the ages of eighteen to sixty years for both males and females have been found satisfactory.

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Cardiovascular System. The existence of cardiac disease will usually disqualify a prospective donor. The person with exertional dyspnea, nocturnal dyspnea, angina pectoris, cardiac arrhythmias which are not physiologic, or pitting edema, should not be bled except in the special instance in which his physician requests it as treatment for cardiac disease. The presence of hypertension, with a systolic arterial pressure in excess of 200 mm. of mercury or a diastolic pressure over 110 mm., is likewise disqualifying, except when bleeding is performed as a therapeutic measure. Persons with a systolic arterial pressure of less than 100 mm. of mercury are not good subjects for blood donation because of their proneness to fainting. Arteriosclerosis alone, except in extreme degree, does not contraindicate bleeding. It should be noted, however, that coronary artery occlusion occurs in persons with this disorder although it is rarely associated with blood donation.[1] All other vascular diseases, including active or recent thrombophlebitis, should exclude the prospective donor.

Hemoglobin Concentration. The prospective donor should not be accepted if the hemoglobin concentration of his blood is low. A level of 12.2 gm. per 100 ml. is generally recognized as satisfactory. The test for hemoglobin concentration can be accomplished rapidly and accurately by the use of the copper sulfate method (p. 218)

Blood Dyscrasias. The existence of any blood dyscrasia, except polycythemia, disqualifies the donor. The blood from the patient with polycythemia may be used for transfusion but its increased viscosity and its tendency to clot render both collection and administration difficult. These obstacles are minimized if the blood is quickly and cleanly drawn into a preservative solution of large volume (Chap. 13).

Transmissible Disease. *Syphilis.* The transmission of syphilis by transfusion has been recorded many times (Chap. 12). Adequate precautions against the acceptance of a donor whose blood is infectious must be taken. A person exhibiting primary or secondary lesions, one receiving antiluetic treatment, or one whose blood gives a positive serologic test for syphilis should not be accepted as a donor. The blood from donors with evidence of syphilis should not be transfused even though preservation is known to minimize the likelihood of survival of spirochetes.

Malaria. It is particularly difficult to determine the suitability for blood donation of a person who has recovered from malaria. The blood from such persons may be intermittently infectious for many years (Chap. 12). Persons who have lived in malarious regions

the acceptance of such persons as donors must rest upon rather arbitrary standards. A tentative plan in some places is to reject persons as donors who have had malaria within two years. Those who have resided in malarious regions are rejected unless they have been symptom-free for at least two years after suppressive drugs have been discontinued.

Hepatitis. One of the most troublesome sequelae of transfusion is hepatitis. As discussed on pages 295 and 356, an icterogenic agent can be transmitted from one person to another by transfusion of whole blood, plasma, or serum. The donor is himself infective before he develops clinical manifestations of the disease. It is probable also that many persons are carriers of the disease without manifesting clinical signs or symptoms. A history of jaundice or febrile disease without jaundice which coincides with the occurrence of infectious hepatitis in close associates should disqualify the donor if the episode has occurred within six months. For the same reason donors should be rejected if they have received transfusions of serum or plasma, or hypodermic injections within six months.

Other Infectious Diseases. Other infectious diseases have been transmitted occasionally by transfusion.[3] In most cases the blood has been collected during the prodromal stage of the disease. The donor is infectious at the time. Safeguards against the transmission of disease include the rejection of donors with a history of exposure, with rhinitis, or with fever.

Allergy. The problem of passive transfer of sensitivity by transfusion and the injection of antigens is discussed in Chapter 12. Fortunately the result of passive transfer is rarely detrimental to the recipient. Care should be taken to exclude donors with chronic allergic disease or drug sensitivity. Those with seasonal allergy should be employed as donors only during the seasons when they are normally symptom-free. Persons with other sensitivity may act as donors, provided the recipient is protected from contact with the allergen for three or four weeks after transfusion.

Blood Group of the Donor. *A-B-O System.* The procedure of choice is to select a donor of the same group in the A-B-O system as the recipient. Transfusion with group-specific blood will prevent most of the occasional reactions from high titer agglutinins. Even in the transfusion of the AB recipient, the universal recipient, this practice should be followed.

Rh-Hr System. When transfusions are contemplated for an isosensitized recipient, an Rh-negative or Hr-negative donor should be employed as indicated. It is also recommended that Rh-positive blood be given routinely to Rh-positive recipients and Rh-negative blood to Rh-negative recipients (Chap. 8).

Cardiovascular System. The existence of cardiac disease will usually disqualify a prospective donor. The person with exertional dyspnea, nocturnal dyspnea, angina pectoris, cardiac arrhythmias which are not physiologic, or pitting edema, should not be bled except in the special instance in which his physician requests it as treatment for cardiac disease. The presence of hypertension, with a systolic arterial pressure in excess of 200 mm. of mercury or a diastolic pressure over 110 mm., is likewise disqualifying, except when bleeding is performed as a therapeutic measure. Persons with a systolic arterial pressure of less than 100 mm. of mercury are not good subjects for blood donation because of their proneness to fainting. Arteriosclerosis alone, except in extreme degree, does not contraindicate bleeding. It should be noted, however, that coronary artery occlusion occurs in persons with this disorder although it is rarely associated with blood donation.[1] All other vascular diseases, including active or recent thrombophlebitis, should exclude the prospective donor.

Hemoglobin Concentration. The prospective donor should not be accepted if the hemoglobin concentration of his blood is low. A level of 12.2 gm. per 100 ml. is generally recognized as satisfactory. The test for hemoglobin concentration can be accomplished rapidly and accurately by the use of the copper sulfate method (p. 218)

Blood Dyscrasias. The existence of any blood dyscrasia, except polycythemia, disqualifies the donor. The blood from the patient with polycythemia may be used for transfusion but its increased viscosity and its tendency to clot render both collection and administration difficult. These obstacles are minimized if the blood is quickly and cleanly drawn into a preservative solution of large volume (Chap. 13).

Transmissible Disease. *Syphilis.* The transmission of syphilis by transfusion has been recorded many times (Chap. 12). Adequate precautions against the acceptance of a donor whose blood is infectious must be taken. A person exhibiting primary or secondary lesions, one receiving antiluetic treatment, or one whose blood gives a positive serologic test for syphilis should not be accepted as a donor. The blood from donors with evidence of syphilis should not be transfused even though preservation is known to minimize the likelihood of survival of spirochetes.

Malaria. It is particularly difficult to determine the suitability for blood donation of a person who has recovered from malaria. The blood from such persons may be intermittently infectious for many years (Chap. 12). Persons who have lived in malarious regions and taken suppressive doses of antimalarial drugs may be infected without exhibiting clinical signs for many months. Examination of blood films does not always disclose the parasites. For these reasons

entry, the direction of the needle may be altered so that the shaft continues up the lumen of the vein. A short needle is best inserted its entire length and a longer one approximately an inch (2.5 cm.) past the point of entrance into the vein.

Speed of Flow. Bleeding should proceed with sufficient speed to prevent coagulation within the apparatus. It is difficult to remove blood at a rate which will affect the donor. Ordinarily between five and ten minutes are ample for withdrawing 500 ml. of blood. A steady flow is preferred rather than an intermittent one. Periods of stoppage are conducive to clotting within the tubing. Collapse of the vein and cessation of flow occur from (*a*) too rapid emptying, (*b*) improper placement of the needle in the vein so that its opening impinges on the vein wall, or (*c*) excessive suction applied to the bottle. The speed of withdrawal, therefore, is largely predetermined by the size of the vein and the caliber of the needle. The velocity of flow should be adjusted to produce a steady stream without collapse of the vein. Strict attention should be given to the position of the needle throughout bleeding.

Aftercare of the Arm. The care of the arm after bleeding should prevent further blood loss, either externally or into the subcutaneous tissues, and promote healing of the puncture wound. Attention to several details will prevent much inconvenience to the donor. The tourniquet must be released before the flow through the apparatus is interrupted and the needle withdrawn. Immediately after withdrawal of the needle, pressure should be applied directly over the puncture site. Doubling the arm of the donor is poor practice unless the donor simultaneously holds a sponge pressed firmly against the puncture site with the other hand. Otherwise the sponge may slip and produce pressure on the vein above the puncture and promote bleeding. A preferable procedure is to elevate the arm sufficiently to collapse the vein. The wound is usually closed in a few minutes. Occasionally seepage of blood continues and a bandage is necessary. This should be applied snugly and is best accomplished with a two inch (5 cm.) roller bandage If iodine has been used on the skin, it should be removed as completely as possible to prevent burning.

PHYSIOLOGY OF BLOOD DONATION

Effects of Bleeding. The rapid loss of 500 ml. of blood is not usually attended by serious alteration in the hemodynamics of the circulation nor is the resulting anemia severe enough to inconvenience the normal subject. Nevertheless, the removal of approximately 10 per cent of the circulating blood does result in definite

TECHNIQUE OF BLEEDING

Choice of Vein. Any suitable vein may be chosen for venipuncture. Usually one in the antecubital fossa is the most convenient and possesses the desirable characteristics. These veins are large anough to permit ready entrance of the needle and allow free flow of blood about the needle. There is stability in the subcutaneous tissues so that the vein does not roll away from the needle. The walls are sufficiently thick that the needle will enter cleanly. Thin walled veins are easily torn with resultant extravasation of blood. Specifically to be avoided are the very superficial bright blue veins found particularly in obese women, which are invariably fragile. Other veins of the forearm may be used, although their mobility may make puncture difficult. The deeper veins are usually more satisfactory although they may not be visible. The transfusionist should train himself to palpate such vessels and distinguish them by touch from other structures such as tendons.

Preparation of the Skin. The skin should be cleaned meticulously. The application of soap and water, followed by 70 per cent ethyl alcohol, is entirely adequate. A routine practice is to paint the skin in the area about the proposed puncture site with tincture of iodine, USP. The iodine is permitted to dry and then removed with 70 per cent ethyl alcohol on sterile gauze sponges.

Anesthesia. Venipuncture with large needles is attended by sufficient pain as to render the use of local anesthesia desirable. For this purpose 0.5 to 1 per cent procaine hydrochloride solution is satisfactory. One-half to 1 ml. will be found sufficient. About 0.25 ml. is injected just subcutaneously and the infiltration continued down to the wall of the vein. Care should be taken not to inject an amount which will interfere with palpation or visualization of the vein. Three to five minutes should be allowed for the action of the anesthetic before the large needle is inserted.

Tourniquet. The venous flow should be obstructed with a tourniquet at a point central to the site of venipuncture. Care should be taken in its application that the arterial flow is not impaired. This is accomplished by adjusting the tension of the tourniquet below that of the diastolic blood pressure. A piece of rubber tubing of suitable length, or a sphygmomanometer cuff, will be found satisfactory for this purpose. The latter may be inflated to a pressure of 30 or 40 mm. of mercury. If tubing is used, the presence of a normal pulse volume in the radial artery may be used as evidence that the tourniquet is not too tight.

Insertion of the Needle. The needle is inserted through the skin directly over the vein, at an angle of 30 degrees with the skin surface. The second step is to plunge it directly into the vein. After

anemia. The time required for the regeneration of hemoglobin after removal of 500 ml. of blood varies with the individual, but the usual period is about eight weeks.[6,7,8,9] Fowler and Barer found that the removal of 555 ml. of blood was attended by a drop of 2.3 gm. of hemoglobin per 100 ml. in the average donor. The average time for hemoglobin regeneration was 49.6 days. Males showed an average increment of 0.049 gm. per 100 ml. per day and females 0.04 gm. The administration of iron salts by mouth shortened the average recovery period to 35.2 days after the first blood donation, but there was progressively less effect after subsequent bleedings. Their studies yielded no evidence, however, that the bone marrow was exhausted after repeated venesection.[8,9] These results clearly indicate that eight weeks is the least allowable interval between bleedings for the average person. As a further precaution the hemoglobin concentration of the donor's blood should be determined before each donation to exclude those requiring a longer than average period for hemoglobin regeneration.

DONOR ACCIDENTS

Although the untoward effects of blood donation are usually transient, some accidents may result in serious illness and a very few are fatal. These complications may be classified as follows: (1) local injury at the site of venipuncture, (2) infection, (3) syncope, (4) cardiovascular accidents, and (5) miscellaneous. Local injury is by far the most common and syncope is second in order of frequency. Although not usually serious, the latter is the most troublesome reaction encountered in donors.

Local Injury

Hematoma. Extravasation of blood at the site of venipuncture is usually of little consequence, producing some discoloration and slight inconvenience to the donor. Boynton and Taylor[1] estimated that 10.1 per cent of donors suffered some degree of this type of complication. Several measures may be taken to prevent its occurrence. Clean entry of the needle into the vein is a prime requisite. This is accomplished by the choice of a suitable vein, inspection of the needle to insure sharpness, and careful attention to technique. Extravasation of blood may occur when flow through the apparatus is interrupted and the tourniquet is left in place, either from clotting in the tubing or from clamping the tubing and not promptly releasing the tourniquet. Proper care of the arm after bleeding will further serve to reduce the incidence of hematomata (p. 237).

changes and calls into action certain compensatory mechanisms. Most studies of these phenomena have been made on individuals subjected to the withdrawal of 900 ml. or more of blood, which is approximately twice the volume usually taken from a donor. The effects observed in these experiments are correspondingly greater but qualitatively similar to those in blood donors. The immediate result of bleeding is to reduce the total blood volume at the expense of both cells and plasma. There is a fall in the pressure in the right auricle and a reduction in the cardiac output.[2]

After bleeding, compensation occurs by vasoconstriction and a slight increase in the heart rate sufficient to maintain the arterial blood pressure in the face of decreased cardiac output. If fainting occurs, it is accompanied by failure of these mechanisms.[3] The second event in recovery is the restoration of blood volume by augmentation of the amount of plasma. According to some investigators,[4] during the first few hours the increment of plasma results from migration of protein-poor fluid into the blood stream. This is followed by the addition of fluid containing protein in the concentration present in normal plasma. Others[5] have found no evidence of the initial phase but believe the entire process consists of addition of fluid with a normal concentration of protein. The ultimate result in either case is an expansion of the plasma volume equal to the amount of whole blood removed. This produces a normal blood volume, decreased cell volume, increased plasma volume, decreased total circulating hemoglobin, augmented total circulating plasma protein, with normal plasma protein concentration and diminished hemoglobin concentration.

The rate at which hemodilution occurs varies remarkably with the circumstance and the individual. In their study on volunteers Wallace and Sharpey-Schafer[5] tabulated the time required for the blood volume to return to normal after withdrawal of various amounts. There was apparently no correlation between blood loss and recovery. The smallest amount of blood withdrawn was 465 ml. after which the recovery period was four hours. The greatest volume removed was 1150 ml. and a normal blood volume was attained after seventy-two hours. However, when approximately the same volume of blood (1000 to 1150 ml.) was removed from thirteen subjects, the time for hemodilution ranged from five to ninety-six hours.

Hemoglobin Regeneration. The recovery of the donor cannot be considered complete until the hemoglobin concentration has returned to the predonation level. This is of very practical importance in relation to repeated bleeding of the same individual. Venesection at too frequent intervals will produce secondary

Variations in the number of faints recorded by these observers may have arisen from different criteria employed for diagnosis. It can be stated that the incidence of syncope in blood donors is about 5 per cent.

Factors Influencing the Occurrence of Faints. *Sex.* Poles and Boycott[11] found that syncope occurred equally in both sexes but others[1, 12, 13] have recorded a greater incidence in females. The reaction seems to be most common in young women between the ages of eighteen and thirty years.

Age. All agree that fainting is more common in young persons of both sexes.[1, 11, 12, 13] The incidence decreases approximately 50 per cent in women over thirty-five years and in men over forty-five.

Relation to Meals. Most observers[11-14] believe that the probability of fainting is increased if the donor has fasted several hours. The critical period is thought to be from three to five hours. Boynton and Taylor,[1] however, were unable to find any statistical correlation between fasting and syncope.

Psychic Factors. Apprehension over giving blood is regarded as an important factor in fainting.[1, 11-13] Persons likely to faint may often be recognized in advance by their unusual interest in the procedure of bleeding or their complete and elaborate disregard for all details. Zukerman[15] and others[1, 14] state that these persons often have a history of frequent fainting.

Body Build and Blood Volume. Several observations have been reported on the relation of body build and blood volume to the incidence of fainting. Boynton and Taylor[1] noted that syncope was more common in persons weighing less than 120 pounds (54.5 kilograms). The tall and thin, as well as the short and thin, have been reported by Williams[12] as more apt to faint. Poles and Boycott[11] stated that a small blood volume predisposes to fainting and expressed the corollary that syncope becomes more likely as larger amounts of blood are withdrawn. They observed only half as many faints when 440 ml. of blood was withdrawn as when a donation of 540 ml was permitted. On the other hand, Moloney *et al* [14] could find no relation between syncope and blood volume.

Menstruation A marked increase in the incidence of fainting was observed by Williams[12] in women bled during menstruation.

Occupation. Office workers and others whose occupation entails a minimum of physical activity exhibit a greater tendency to faint than those whose daily life is more strenuous.[1, 13]

Number of Donations. Donors who return for a second bleeding after their first experience are not usually subject to fainting.[1] A natural process of selection seems the most likely explanation.

Venipuncture. The pain of venipuncture has been considered by

Dermatitis from Adhesive Tape. Some persons develop a contact dermatitis from adhesive tape. Those known to be sensitive should have some other type of bandage applied. All donors should be instructed to remove the tape after six or eight hours.

Dermatitis from Iodine. Failure to remove the iodine used in the preparation of the skin for venipuncture may result in vesicle formation and desquamation. Very rarely an individual is found to exhibit an idiosyncrasy to iodine which is manifested by a severe reaction with fever and a generalized skin eruption.[10]

INFECTION

Abscess and Cellulitis. Venipuncture produces a portal for possible entry of bacteria into the subcutaneous tissues. Occasionally a localized infection results. This may be either an abscess or cellulitis of the arm.[1]

Thrombophlebitis. Just as the needle puncture may permit ingress of bacteria subcutaneously, so may it provide an entry into the vein. The result may be a thrombophlebitis, localized within a single vein or spreading to contiguous vessels. More rarely a generalized migratory thrombophlebitis develops.[1]

SYNCOPE

Incidence. Fainting has been variously reported to occur in from 2.9 per cent[11] to 6.8 per cent[12] of donors. Boynton and Taylor[1] stated that 8.9 per cent of 40,000 donors responding to a questionnaire answered affirmatively to the question "Did you have any ill effects immediately following the donation?" In view of the experience of others it seems likely that these donors must have considered symptoms not related to syncope in making their replies. In five reports [11, 12, 13, 14, 15] the total incidence of observed fainting was 4.6 per cent in 46,500 donations. The data are summarized as follows:

Incidence of Syncope in Blood Donors

Authors	*Number of Donors*	*Number of Faints*	*Per Cent*
Poles and Boycott[11]	10,723	310	2.9
Greenbury[13]	5,897	291*	4.9
Williams[12]	3,241	222*	6.8
Maloney *et al.*[14]	16,133	689	4.2
Zukerman[15]	10,506	659	6.2
Totals	*46,500*	*2171*	
		Average	*4.6*

*Calculated from the percentage given

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some observers[14] as an infrequent cause of syncope. The careful use of a local anesthetic does much to reduce reactions from this cause.

Symptoms. Syncope in blood donors has essentially the same features as that seen under other circumstances. In many persons there is a definite prodrome of a feeling of warmth, particularly in the epigastrium, weakness, and dizziness. If the reaction progresses, there follows nausea, blurring of vision, and shortness of breath. *Objectively, pallor, sweating, fall in blood pressure, slowing* of the pulse, and dilatation of the pupils may be noted. Finally there may be vomiting, loss of consciousness, urinary and fecal incontinence, muscular twitching or generalized convulsions. Occasionally hyperventilation and tetany are encountered.[1, 14–16] There is little agreement as to the frequency of these signs and symptoms. Pallor, sweating, and loss of consciousness are among the most common. Pallor is almost universal and sweating is noted in about two thirds of the cases. Loss of consciousness has been reported in as few as 11 per cent[1] and as many as 95 per cent[14] of the cases. Generalized convulsions were noted in 28 per cent of the cases by Moloney *et al.*,[14] 3.4 per cent by Boynton and Taylor, and in 0.3 per cent by Zukerman.[15]

Treatment. In the early stages, an attack of syncope often may be aborted by having the donor lie flat with the head horizontally. If the reaction progresses, the bleeding should be discontinued. Usually a few minutes' rest in the supine position will result in recovery. After ten or fifteen minutes the donor may be permitted to sit up. Any return of symptoms indicates need for further rest. If symptom-free, walking should be encouraged under close supervision until complete recovery is assured. Warm tea or coffee, with perhaps a cracker or biscuit, seem to promote recovery. Hot beverages, however, are contraindicated as the resulting vasodilatation in the stomach seems to induce fainting. Aromatic spirits of ammonia USP may be given in 2 ml. doses in water orally, or inhaled. Zukerman[15] stressed the importance of early ambulation as a factor in recovery. Occasionally a person faints each time rising is attempted and this state may persist for several hours. Boynton and Taylor[1] found that hospitalization was necessary in a very few instances, and even the reinjection of blood on several occasions.

Prevention. The occurrence of syncope in donors may be reduced somewhat by the exclusion of those with neurotic complaints and those who have fainted frequently on other occasions.[15] *A cheerful matter-of-fact manner in the persons carrying* out the bleeding will do much to allay the apprehension which seems so often to be the precipating factor. The approach to the donor

should be friendly and he should be made to feel that his donation is being individualized. Constant engagement of the subject in conversation by the doctor or nurse is also of value.[12,14] It is suggested that arrangements be made to bleed donors with the utmost dispatch after they present themselves so that they will not have an opportunity to wait in idleness which engenders more apprehension. It is also advisable to keep the bottles of blood from the view of the donors at all times as the sight of their blood in a flask seems to initiate symptoms in some.

Delayed Syncope. A certain number of donors, fortunately small, exhibit this dangerous complication. There may be a return or the initial appearance of symptoms several hours after bleeding. The reaction may take place in the hospital corridors, on the street, in the home, or at his place of work after he has been released from medical observation. Under such circumstance syncope may result in serious or fatal injury. Poles and Boycott[11] believe that delayed fainting is due to a transient orthostatic hypotension.

Injury During Syncope. The donor may fall and be injured. The most common injuries are scalp and head lacerations, abrasions, and mild cerebral concussion. Fracture of the skull, facial bones, clavicle, fingers, and olecranon process have been encountered.[1]

Epidemic Fainting. An interesting phenomenon which can be due only to suggestion has been noted many times.[12 14] This is syncope which appears in several consecutive donors early in the course of bleeding, or even as the person awaits his turn to be bled. These "epidemics" are usually initiated by syncope in one member of the group or by a minor incident such as the sight of a bottle of blood. It occurs most frequently in a group of persons from one family or in a group of acquaintances who appear together to give blood. It is also more frequent in younger persons and particularly females.

Cardiovascular Accidents

Cardiovascular accidents during or soon after bleeding are noteworthy chiefly because of their infrequency. In an estimated 3,500,000 donors Boynton and Taylor[1] collected eight instances of death from coronary insufficiency and two from cerebral thrombosis. In all but one (a case of cerebral thrombosis) the onset of symptoms occurred within twelve hours after bleeding. These observers also recorded two cases of angina pectoris, one of coronary thrombosis, and one of cerebral thrombosis with the onset during donation. There were three instances of cerebral thrombosis and

one of cardiac failure with symptoms which appeared within two hours after donation. All eight patients recovered. Zukerman[17] reported a case of coronary insufficiency in a man of forty-three years who died shortly after giving blood. In five of ten fatalities cited by Boynton and Taylor[1] there were preexisting symptoms of cardiovascular disease which were denied by the donors before bleeding.

Air Embolism

The accidental injection of air into the donor's vein has been occasionally reported, with fatal outcome in at least one case.[18-20] This usually results from either defective equipment or improper *understanding of the operation of the donor apparatus.*

Perhaps the simplest example is in the case of gravity flow collection equipment. Three factors are usually necessary: (1) the air vent becomes plugged, making the apparatus a closed system; (2) the chilled bottle of preservative solution is warmed by the room air and by the blood running into it and, as a result, the air in the bottle expands; (3) the expanding air is displaced into the vein by blood running into the flask.

When vacuum bottles are used for collection, the mechanism is slightly different in some of the details: (1) the vacuum is inadvertently lost by improper manipulation of the apparatus, by leakage through the connections, or the flask was improperly evacuated; (2) the chilled bottle of preservative solution is warmed by the room air and the blood running into it, causing the enclosed air to expand; (3) the expanding air is forced into the donor's vein by displacement from the flask by blood.

These accidents can be avoided by the constant vigilance on the part of the transfusionist who is fully informed as to the mechanics of this type of accident. The flow of blood should be constantly watched. Valves on evacuation bulbs on gravity flow apparatus should be tested and arranged so that there is no possibility that they can be reversed. Vacuum type of collection bottles can be tested for the presence of at least some degree of vacuum by striking the bottle with the palm of the hand and eliciting the characteristic click produced by fluid in the vacuum. The tubing and valve connections in vacuum equipment should be particularly tight at all times and should be replaced at the least sign of loose-fitting.

The treatment of air embolism is to place the patient horizontally, lying on the *left* side and possibly to administer oxygen by inhalation until the embolus has been absorbed. For a fuller discusssion consult page 290.

Tetany

Hyperventilation tetany occurs in a very few donors as a result of apprehension. It is usually observed in a person donating blood for the first time and is more commonly encountered in women.[11] The excited subject overbreathes, producing a lowered alveolar and arterial concentration of carbon dioxide, with resultant alkalemia.[11] If allowed to progress, Chvostek's sign and carpopedal spasm develop. The incidence of tetany is very low. Moloney *et al.*[14] reported six cases in 16,133 donors. The condition is treated by the administration of carbon dioxide by inhalation, either directly or by rebreathing. A paper sack may be placed over the nose and mouth and usually produces quick recovery. Sometimes rebreathing fails and calcium gluconate should then be injected intravenously.

Arterial Thrombosis

One case of multiple arterial thrombosis has been reported[17] which occurred in a professional donor after bleedings of 375 and 500 ml. of blood in a five day period. No clear-cut causal relationship could be demonstrated but the possibility could not be disproved.

Reactions to Procaine

Theoretically there is no reason why procaine reactions should not be encountered in blood donors since 0.01 gm. is sufficient to produce systemic effects in a sensitive person.[10] The use of the tourniquet should afford protection. No such cases of toxicity have been reported.

THE UNIVERSAL DONOR

Hemolytic Reactions. It has been assumed in the past that blood of group O may be transfused into the circulation of a recipient of any other group with impunity, because the cells are not clumped by anti-A or anti-B agglutinins and the donor's incompatible agglutinins are sufficiently diluted in the recipient's circulation to be impotent. Unfortunately this has been proven not to be entirely correct. If the agglutinin titer of the donor's group O blood is sufficiently high, the A, B, or AB cells of the heterologous recipient may be agglutinated and hemolysis result. A few cases which illustrate this phenomenon have been reported in the literature.

Employment of a universal donor, however, is justified when lack of time or laboratory facilities in an emergency precludes the search for group-specific compatible blood. For this reason it is useful to

obtain a perspective on this type of reaction. Very few transfusions of universal blood result in serious sequelae in recipients of other groups. The obvious explanation is that few group O bloods contain a sufficiently high titer of agglutinins. Two facts which should be known are the agglutinin titer which is dangerous to transfuse and the proportion of group O bloods which contain this dangerous titer.

The titer of agglutinins in group O blood which is definitely dangerous is unknown. Unfortunately, different persons measure titer by methods which are not strictly comparable (p. 127). During World War II the American Red Cross accepted as safe an agglutinin titer of 1/600.[26] Later Tisdall *et al.*[28] found agglutinin titers of more than 1/200 to be dangerous. Ebert and Emerson[27] concluded that the transfusion of blood containing agglutinins with a titer of more than 1/500 was apt to be associated with hemoglobinemia and hemoglobinuria. There is no doubt that this disagreement in titers arises not only from differences in the technique of titration but also from inherent faults in the procedure. Even though no definite titer may be proved to be dangerous, the use of a standard reference serum (p. 127) would determine approximately the number of bloods which could be expected to produce hemolysis from transfusion. For a further discussion of observations in plasma infusions consult page 354.

Ebert and Emerson[27] found that 0.5 per cent of group O donors had agglutinins with what they regarded as a dangerous titer. But the susceptibility to reactions of recipients transfused with group O blood seems to vary. Tisdall *et al.*[28] observed that hemoglobinuria occurred in transfusions of plasma with titers of 1/600. Other subfacts in their study received plasma with much higher agglutinin titers without exhibiting evidence of gross hemolysis. The variables encountered in this type of study make the determination of incidence of hemolysis difficult, but the comparison of two series of transfusions by one of the authors[28] is of interest. In 7299 transfusions of homologous blood the incidence of reactions of all types was 4.22 per cent of which 0.20 per cent were hemolytic. In 9392 transfusions of heterologous blood there was an incidence of reactions of all types of 4.80 per cent with 0.18 per cent hemolytic reactions. The difference was not considered significant. Nevertheless hemolytic reactions from the transfusion of group O into recipients of other groups may cause hemolytic reactions and even death.

Other Effects of Transfusion of Universal Blood. *Destruction of Recipient's Red Cells.* Some evidence has been reported that the erythrocytes of groups A, B, or AB may be destroyed by the transfusion of group O blood, although frank hemolysis is not apparent. Tisdall

et al.[26] transfused volunteer recipients with plasma containing anti-A and anti-B agglutinins in high titer. Thirty-four of thirty-nine subjects showed a marked increase in the bilirubin content of the blood plasma, although only eight had hemoglobinuria. Similar observations were made by Ebert and Emerson[27] and by Aubert *et al.*[28] The cited experiments of Tisdall included the administration of plasma with agglutinin titers ranging from 1/500 to 1/2000 to four volunteers. In two the concentration of free hemoglobin in the recipient's plasma attained a value of 10 mg. per 100 ml. Of seven subjects of group A or B who received 1000 to 1560 ml. of group O blood, three showed significant reduction in the total volume of erythrocytes in forty-eight to seventy-two hours. The agglutinin titer of these bloods was not stated. Repeated transfusions of pooled plasma, after the initial whole blood transfusion, increased the destruction of the recipient's cells in three instances.

Increased Fragility of Recipient's Erythrocytes. Ebert and Emerson[27] observed increased osmotic fragility of the recipient's erythrocytes after repeated administration of universal blood and pooled plasma to burned patients. This led to the transfusion of twelve other patients of group A Rh positive with group O blood. Seven who received blood having an agglutinin titer of more than 1/200 showed increased osmotic fragility of the red cells.

Anti-O Agglutinins. The serum of some persons belonging to groups A_1 and A_1B contain the irregular agglutinin anti-O (or anti-A_2) which reacts with group O cells (p. 55).[24,25] Usually the titer is low and it is more active in the cold. However it must be considered at times as a possible source of reactions to universal blood.[26]

Neutralization of Anti-A and Anti-B Agglutinins. The isolation and commercial preparation of the A and B substances (p. 57) has made possible the neutralization of the agglutinins in universal blood and plasma. It has been recommended that 25 ml. of 1/1000 solution of A substance (25 mg.) and 10 ml. of B substance (10 mg.) be added to each 500 ml. of group O blood which is used as universal donor blood.[30] This markedly reduces the agglutinin content of the average group O blood. Blood with agglutinins of high titer would not necessarily be rendered safe by this procedure.

PLACENTAL BLOOD

Placental blood, collected at the time of delivery, has been found satisfactory for transfusion when proper preservation and precautions against bacterial contamination have been taken.[31] An average volume of 125 ml. has been obtained when the umbilical cord is clamped immediately after delivery.[32] Modern practice in trans-

fusion renders this source of blood of little value because of the small volumes obtained and the difficulty of avoiding bacterial contamination. Only a negligible portion of the total need for blood in a general hospital could be satisfied in this manner. In addition it has been demonstrated that the procedure is detrimental to the infant because it deprives the fetal circulation of a large proportion of its blood volume.[33] This source of blood cannot be recommended.

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CHAPTER 11

The Recipient and the Administration of Blood

BY ROBERT C. HARDIN

TECHNIQUE
DOSAGE AND RATE OF FLOW
INJURY TO THE RECIPIENT
PHYSIOLOGY OF BLOOD TRANSFUSION

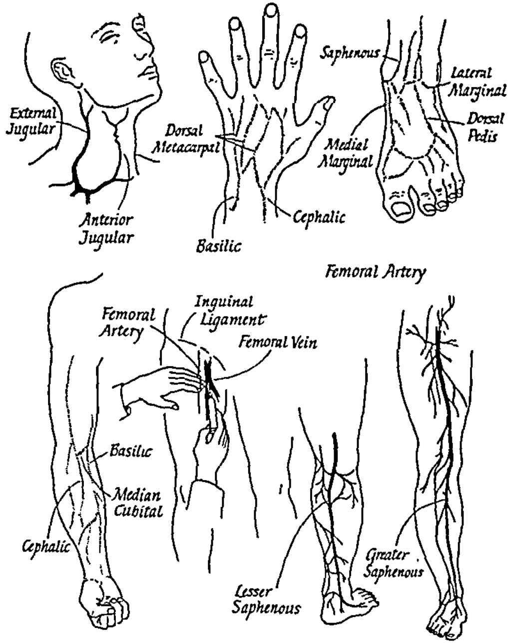
The obvious benefits derived by patients suffering some correctable defect of the blood have led to an ever increasing use of blood transfusion. The greatest impetus came from the classification of humans into four blood groups (A-B-O system) and the explanation of serious reactions on that basis. Two other discoveries, the delayed method of transfusion and techniques for blood preservation, have simplified the procedure of transfusion and widened its applicability. Other advances, such as the discovery of the Rh factor and the explanation of pyrogenic reactions, have increased the safety with which blood may be administered. Because of these factors blood transfusion is now commonly employed in the treatment of a wide variety of diseases. The relative infrequency of serious consequences may lead the physician to consider that blood transfusion is an innocuous procedure. He will, however, be rudely awakened because transfusion is attended by a very real morbidity and a definite mortality. These facts should be kept uppermost in mind when the advisability of transfusion in a given case is being considered. The probable benefits must always be weighed against the infrequent but possible untoward results. *Blood transfusion in the past has proved fatal in 0.14 per cent of cases.*[1] If no other fact is considered, this is sufficient warning. The death rate following the surgical treatment of uncomplicated, acute appendicitis is not appreciably higher. Yet considerably more time and thought are commonly devoted to advising operation in appendicitis than are employed in considering transfusion. The procedure can be justified only when the blood is carefully administered to a patient in whom the inherent dangers are overshadowed by the expected benefits.

TECHNIQUE

EQUIPMENT

Satisfactory equipment is the first requisite of successful transfusion. Discussion and illustration of the several types are found in Chapter 26.

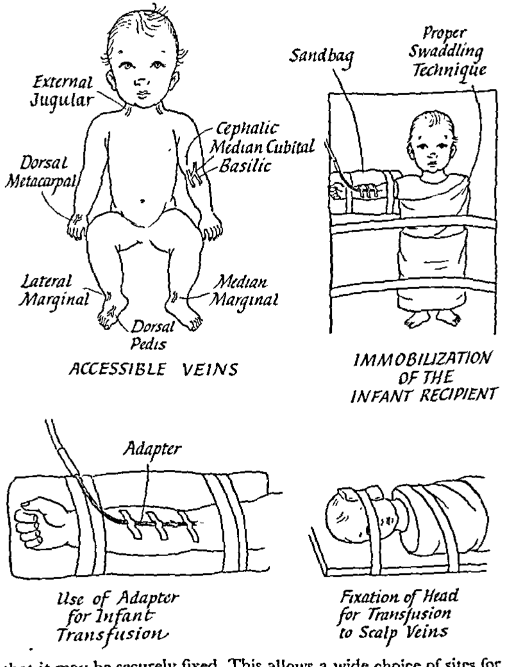
VEINS ACCESSIBLE FOR TRANSFUSION IN THE ADULT RECIPIENT



ROUTES OF ADMINISTRATION

Intravenous. *Choice of Vein.* Blood or plasma may be injected into any vein large enough to admit the needle for a sufficient distance

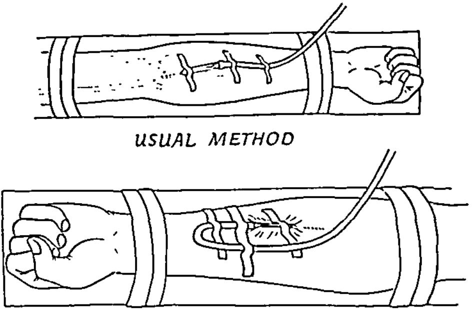
SPECIAL TECHNIQUES IN THE TRANSFUSION OF INFANTS



that it may be securely fixed. This allows a wide choice of sites for transfusion. The diagram on page 251 depicts the commonly accessible veins in the adult. Most transfusions are given into the

veins of the antecubital fossa. However when these are not available because of injury, desire to avoid operative sites, or previous destruction by canalization or thrombosis, others may be used with equal facility. In infants the veins of the dorsum of the hand are frequently utilized. The ordinarily available veins in infants are shown in a diagram on the opposite page.

FIXATION OF NEEDLE



USUAL METHOD

FIXATION TO PERMIT MOBILITY

Venipuncture. The technique of venipuncture for admininstration is the same as that for bleeding (p. 236). The needle must be firmly secured and the tubing so fixed that minimal movement of the extremity does not dislodge the needle. It is important that the entire shaft of the needle be inserted through the skin and that as much as possible be in the vein. The illustration on this page shows a satisfactory method of securing a needle in place and a second method whereby considerable motion of the forearm is allowed.

Femoral Vein. Puncture of the femoral vein may be accomplished by a rather long needle (2½ inches or 6 cm.) and a syringe. The femoral artery is located by palpation just below the inquinal ligament and is marked by maintaining a finger over the site. The needle is then inserted on the medial side of the artery in a plane parallel to it. The syringe is held at an angle of 60 degrees with the skin and the needle slowly inserted, maintaining these relationships until the vein is entered. (Diagram on p. 251.)

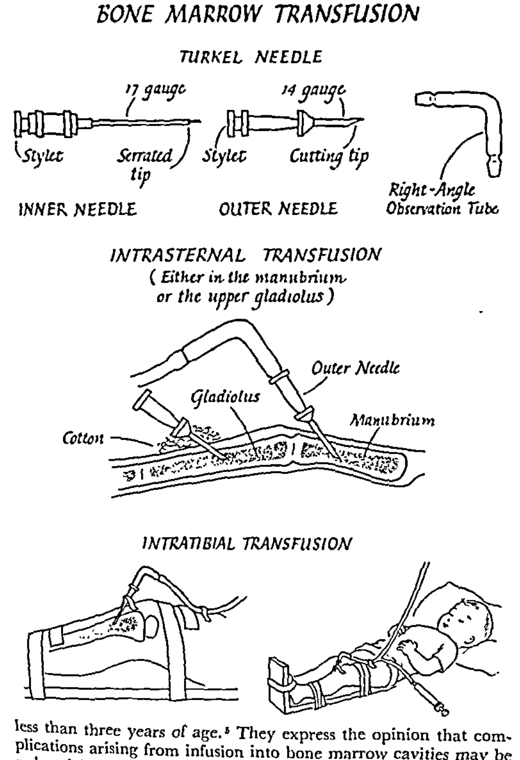
Corpus Cavernosum. Citrated blood or plasma may be injected into the corpus cavernosum of the penis, but the administration of irritating solutions by this route is to be avoided. The needle is inserted obliquely through the skin into the corpus cavernosum, taking care to avoid the dorsal and urethral vessels. More pressure than usual is needed for transfusion because of the resistance to flow within the corpus cavernosum.[2] This site for transfusion cannot be regarded as one of choice but should be reserved for those instances when no other is apparently accessible.

Superior Longitudinal Sinus. Fontanel puncture for transfusion in the infant is enticing because of its ease. The likelihood of thrombosis is slight but the dangers of hematomata are so grave that this route must be condemned.

Umbilical Vein. In newborn infants the umbilical vein is a convenient route for the administration of blood. Because the cord early becomes bloodless and collapses some hours after birth, its usefulness as a site for transfusion is largely limited to cases of prematurity, prolonged labor, fetal hemorrhage, and erythroblastosis. The blood for transfusion is collected in a citrated syringe. The cord is clamped and may or may not be severed. Using a short-beveled needle, the umbilical vein is entered and the blood injected.[3]

Bone Marrow Cavity. The use of marrow cavities as a route for transfusion was well described by Tocantins and O'Neill in 1941.[4] They stated that suitable sites were the sternum, the tibia, and the femur. The latter two are used in children of less than three years because of the undeveloped state of the sternal marrow cavity. The needle, heavy gauge (No. 16 or 17) and of suitable length, is inserted into the central portion of the manubrium or into either the upper and lower portions of the gladiolus. In the femur the site of puncture is anterior and 2 or 3 cm. above the condyle. In the tibia it is anteromedial and 2 or 3 cm. below the condyle. Anesthesia is produced by procaine solution which is injected so that the periosteum is included. In puncture of the sternum the transfusion needle is inserted perpendicular to the skin until bone is struck. Its direction is then altered to an angle of approximately 30 degrees, with the point directed toward the patient's head. The needle is then forced through the anterior plate of the sternum. A syringe is attached and suction applied. If the needle is properly placed, marrow will be aspirated. The needle is then washed out with a few ml. of saline solution and the transfusion apparatus quickly attached. The technique of inserting a needle into the marrow cavity of the long bones is the same except that the point is directed toward the diaphysis. Tocantins and O'Neill have emphasized that (1) this method is indicated only when veins are not accessible, (2) the operator should

be familiar with the technique, (3) no irritating substances should be infused, (4) the route is not to be used when extensive infection is present, and (5) the sternum should not be utilized in patients



less than three years of age.[5] They express the opinion that complications arising from infusion into bone marrow cavities may be reduced by close adherence to these indications and limitations.

Rates of 4.2 ml. per minute into the femur and 25 ml. per minute into the sternum have been attained.[4]

Infusion into bone marrow cavity has been attended by serious complications. Quilligan and Turkel,[6] in a review of the literature, were able to collect six cases of osteomyelitis, five of mediastinitis, one of subcutaneous abscess and one of arterial thrombosis. Tocantins and O'Neill[5] also point out the danger of injury to the epiphysis in long bones as well as that of filling the knee joint with fluid or extravasation into soft tissues as the result of careless technique. *Particular care must be exercised not to puncture the posterior plate of the sternum producing hematoma of the mediastinum and cardiac tamponade.* Specially constructed needles designed to ensure clean entry into marrow cavities and to guard against too deep penetration have been devised.[6, 7]

Intraperitoneal. Animal experiments have shown that erythrocytes introduced into the peritoneal cavity gain access to the circulating blood.[8] This route has been utilized for transfusion, particularly in infants, because of its comparative technical simplicity.[9] However, absorption of blood through the peritoneum occurs too slowly for this method to be useful in those patients requiring rapid replacement of blood volume. Certain other disadvantages have been described.[10, 11] In acutely ill and debilitated patients absorption is likely not to occur and necropsy has shown that almost all of the blood so administered is still in the peritoneal cavity four or five days after transfusion. In addition there is the danger of intestinal perforation.

Special Considerations in Infants

The difficulties encountered in transfusing infants by the intravenous route are mainly those of immobilization and venipuncture. In babies less than a year old the scalp veins are usually satisfactory. The child should be "mummied" and fastened to a firm surface, such as a bedside table top, by the use of adhesive tape. The scalp over the chosen vein is shaved if necessary and cleansed in the usual manner. A hypodermic needle (25 gauge), attached to a syringe containing a few ml. of normal saline solution, is used for venipuncture. When the vein is entered saline solution is injected slowly to test whether the needle is properly placed. When this is demonstrated the transfusion apparatus is quickly attached.[12] Veins of the dorsum of the hand foot may be utilized also. Methods of immobilizing the child for transfusion are shown in the diagram on page 252.

An aid in maintaining the needle in place in the small, fragile veins of infants is the use of small caliber, light weight [illegible] in

transfusion apparatus. Regular recipient sets may be utilized for transfusion in infants by attachment of an adapter tubing of light weight, such as that designed by M. Dick and R. L. Jackson which has been in use for several years in the Pediatrics Department of the State University of Iowa Hospitals.[18] This is shown in the diagram on page 252.

DOSAGE AND RATE OF FLOW

Errors in dosage and rate of flow may be of serious consequence and result in fatality (Chap. 12). The proper volume to be transfused can be calculated accurately and usually one may be guided also by the progress of the patient. Factors to be considered are the age and size of the recipient and the purpose of the transfusion. In most instances blood is administered either to increase blood volume or to augment the erythrocytes. In other words, the purpose of transfusion is usually to combat shock or to treat anemia. The dosage varies both with the disease and with its severity. Equal in importance to the total volume of blood administered is the rate at which it is injected.

Dosage and Rate in Shock. The dosage and rate of administration in the treatment of shock are discussed fully in Chapter 3. A brief summary may be presented here. In secondary shock the best clinical measure of the degree of blood volume depletion is the blood pressure. A systolic arterial pressure below 85 mm. of mercury indicates a loss of at least 25 per cent of the blood volume. In the average adult this is in the neighborhood of 1500 ml. The first 500 ml. of blood should be injected in a minimum of fifteen minutes. In severe cases this may be accomplished in five minutes. The initial 1000 ml. should be administered within thirty minutes. A satisfactory response to transfusion raises the arterial systolic pressure by 10 to 20 mm. of mercury for each 500 ml. transfused. When a systolic pressure of 90 mm. of mercury is attained, the rate of transfusion may be reduced to 10 to 20 ml. per minute until the blood pressure is normal. The rate is then further slowed to 3 to 5 ml. per minute until the circulation is stabilized or the initial treatment of the underlying disease is completed.

Dosage in Anemia. The obvious reason for transfusion in the treatment of anemia is to replenish quickly the erythrocyte volume. The problem arises in the preparation of the anemic patient for operation, in combatting an infection, in the treatment of aplastic anemia, or in those cases of primary or secondary anemia exhibiting a dangerously low erythrocyte count (less than 1,000,000 per cu. mm.). The dosage of blood varies according to the severity of

the anemia and with the rapidity of rise in erythrocyte volume and hemoglobin concentration desired. Marriott and Kekwick[14] have suggested certain goals in the transfusion of such patients. They believe that the anemic patient facing operation should receive sufficient blood to elevate the hemoglobin to 80 per cent, which corresponds to an erythrocyte count of 4,000,000 per cu. mm. For maintenance of blood levels in aplastic anemia they suggest a *hemoglobin concentration of 60 to 80 per cent. This is the equivalent* of 3,500,000 erythrocytes per cu. mm., which will be found entirely satisfactory. It is their practice to administer blood in patients with infection until the erythrocyte volume is normal (hemoglobin 100 per cent). Finally they would transfuse the dangerously anemic patient until a hemoglobin concentration of 45 per cent is attained (an erythrocyte count of 2,250,000 per cu. mm.). In children Halbertsma[15] has calculated that a rise of one million per cu. mm. in the erythrocyte count may be expected from the transfusion of 15 ml. per kilogram of body weight. Mayes[4] has used 10 ml. per pound (22 ml. per kilogram) in the newborn. Calculation of the amount of blood necessary to produce a given rise in hemoglobin concentration or erythrocyte content may be very simply accomplished by using average normal values for blood volume, erythrocyte count and hemoglobin concentration. Thus if a man weighing 154 pounds (70 kilograms), having a red cell count of 3 million per cu. mm. and 9 gm. of hemoglobin per 100 ml. (60 per cent), is to be treated so that a level of 4 million cells and 12 gm. (80 per cent) is attained, the following estimation may be made. The blood volume may be calculated by using the value of 90 ml. per kilogram of body weight.[16] The dose of blood required equals the blood volume multiplied by the desired rise in erythrocyte content per cu. mm. divided by the normal red blood cell count. That is:

$$\frac{70 \times 90 \times 1{,}000{,}000}{5{,}000{,}000} = 1260 \text{ ml.}$$

Marriott and Kekwick[14] utilize the percentage of hemoglobin in the same formula.

$$\frac{\%\ \text{rise in Hb. required} \times \text{patient's blood volume}}{100}$$

Calculating with the value of 40 ml. per pound of body weight as the blood volume, this becomes

$$\frac{20 \times 154 \times 40}{100} = 1232 \text{ ml.}$$

Kilduffe and DeBakey[1] based estimations of dosage on the fact that 0.01 of the blood volume is equivalent to 1 per cent of total hemoglobin. Thus in the hypothetical case under consideration a 1 per cent rise in hemoglobin would be produced by the administration of 63 ml. of blood. The desired 20 per cent augmentation would result from the administration of 63 × 20, or 1260 ml. These calculations are expressions of the same ratio and the process may be extended to include the use of grams per 100 ml. of hemoglobin. Thus the patient in question would require an elevation of 3 gm. per 100 ml. and the dose of blood may be found as follows:

$$\frac{70 \times 90 \times 3}{15} = 1260 \text{ ml.}$$

A useful rule of thumb in estimating the volume of blood to be transfused is that the administration of 500 ml. may be expected to produce a rise of approximately a half million red cells per cu. mm.,[1] which is the equivalent of 1.5 gm. of hemoglobin per 100 ml. (10 per cent) in the average adult. The total volume of blood necessary in a given case may be calculated by any of these methods from one of the usually determined measurements of blood constituents. Table XVIII reiterates the desirable levels to be achieved in the treatment of anemia.

TABLE XVIII

Blood Levels to Be Attained by Transfusion in Various Conditions

Disease Conditions	Erythrocytes (millions per cu. mm.)	Hemoglobin (gm. per 100 ml.)	Hemoglobin (%)
Preparation for Operation	4.00	12.00	80
Aplastic Anemia	3.50	10.50	70
Infection	5.00	15.00	100
Dangerously Low Anemia	2.25	6.75	45

Rate of Flow in Anemia. The time required to achieve the desired levels of erythrocyte content of blood in cases of anemia varies with two factors. One of these is the maximum rate at which the blood may be administered without overloading the circulation. The other is the maximum volume which may be transfused at any one time. These two factors are closely related since small volumes may be given at a fast rate but larger ones must be injected much more slowly. The problem resolves itself into determination of the necessary dosage of blood in a given patient and whether that volume may be given in one injection,

Marriott and Kekwick[16, 17] have advocated continuous drip transfusions of large volumes of blood at a very slow rate to quickly overcome severe anemias. The rate of flow which they advise is 1 ml. per pound of body weight per hour (0.45 ml. per kilogram), except in cases of cachexia, cardiac disease, respiratory disease or severe anemia (hemoglobin of less than 25 per cent). In these instances the prescribed rate is reduced to 0.5 ml. per pound per hour. In addition, it is their practice to divide the transfusion into two parts separated by forty-eight hours when a rise of hemoglobin of 33 per cent or more is to be attained. It will be seen that to raise the erythrocyte count from 1 million to 2.25 million in an average man will require a minimum of ten hours. If more than 33 per cent of the hemoglobin is to be replaced, at least four days must be occupied in this therapy. The same end may be reached by employment of daily administration of smaller amounts, particularly when concentrated erythrocyte suspensions are used. By the latter method approximately 15 per cent of the erythrocytes may be replaced daily with safety.

It is of practical interest therefore to consider the rate at which 500 ml. of blood may be administered. Sharpey-Schafer and Wallace[18] studied the effect of intravenous administration of saline solution (0.9 per cent), serum, and blood at very fast rates on the venous pressure, vital capacity and diastolic size of the heart in subjects with normal cardiovascular systems. In their experiments with protein fluids (serum and blood) they injected from 700 ml. to 2100 ml. at rates varying from 54 to 154 ml. per minute. In all of ten subjects elevations of venous pressure were seen, ranging between 4 and 11 cm. of water. There was slight reduction of vital capacity. In two patients in which it was measured, the diastolic size of the heart was increased. In three of seven, an electrocardiogram showed right heart stress. In general these changes were more marked with the higher rates of transfusion and the larger volumes transfused. The effects were transient. These experiments exceeded the conditions of transfusion in the therapy of anemia but serve to indicate the upper limits of safety. In fact such velocities cannot be attained unless the blood is injected under pressure.

The use of the gravity method of infusion and employment of a 20-gauge needle will automatically limit the speed of transfusion to a rate not in excess of 20 ml. per minute when preserved blood is used. Kilduffe and DeBakey[1] regard 60 ml. per minute as a satisfactory rate for administration of 500 ml. of unmodified blood. In our own experience no ill effects have been noted from the injection of 1200 ml. of preserved blood mixture

at the rate of 40 ml. per minute.[19] A rate of 20 ml. per minute is safe and allows the injection of 600 ml. of blood mixture in thirty minutes, or of larger volumes of preservative mixtures in approximately an hour. There is no reason why an hour and a half to two hours may not be utilized to administer a transfusion, thus increasing the margin of safety. Extension of the time required for injection beyond this period by reduction of rate of flow probably does not increase the safety of the procedure. In the transfusion of patients with cardiac disease it is suggested that blood preservative mixtures of small volume or concentrated erythrocyte suspensions be used. A rate of 1 ml. per minute has been advised[1] in these cases. Of more value is the employment of a small total volume (300 ml.) which may be accomplished by the use of concentrated erythrocytes. Constant observation of the patient for signs of increased venous pressure must be maintained.

Transfusion in Severe Anemia. Certain physiologic abnormalities in cardiovascular dynamics render the transfusion of a severely anemic patient a special problem. These have been the subject of a series of interesting studies at the London Post Graduate Medical School.[20-21] The changes consist of a rapid circulation and arteriolar vasodilatation, decreased blood volume to levels as low as 2 liters, increased right auricular pressure, increased cardiac output and increased percentage of oxygen utilization. In the normal individual, intravenous infusion raises the right auricular pressure and consequently increases cardiac output. Severely anemic patients, however, often exhibit a fall of cardiac output in spite of further increase of right auricular pressure. This phenomenon may be accompanied by the appearance of pulmonary edema. These workers suggest that the heart of the anemic patient behaves like Starling's overloaded heart-lung preparation in that increased venous filling pressure results in falling cardiac output. The practical aspects of these observations may be briefly summarized in the statement that *the circulation of the severely anemic patient is easily overloaded.* It is interesting to note that transfusion in these patients often produces a rise in arterial blood pressure in spite of failing cardiac output. This apparently results from increase in peripheral resistance and cannot be taken as evidence of an improved circulation. In transfusing such patients the physician is well advised to proceed with utmost caution. Small volumes of blood should be given at a slow rate and signs of increased venous pressure should be watched for. It is also suggested that digitalis may be of value to combat rising venous pressure.[22] A dose of 1.5 mg. of digoxin [intr]avenously has been successfully employed.

[C]oncentrated Suspensions of Erythrocytes. Frequent mention

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These determinations should be made as soon as possible if the recipient has a chill or exhibits hyperpyrexia during transfusion, particularly in the early part. In addition, examination of the urine for hemoglobin should be made. For this purpose the urine should be collected routinely in the twenty-four hours after transfusion. Lastly the record of the patient's temperature should be examined, even though he have no complaints. If these procedures are followed after each transfusion few reactions will go unnoted and unexplained.

PHYSIOLOGY OF BLOOD TRANSFUSION

The intravenous injection of blood or plasma into a subject with a normal heart produces exactly the opposite effect of hemorrhage. The immediate results are increased venous and right atrial pressure, increased ventricular inflow, and increased cardiac output. There begins concomitantly, however, an adjustment of the circulation and the blood of such nature that both are brought toward the normal state. The exact nature of this adjustment depends largely upon the underlying deficiency for which the transfusion was given.

Changes in Cardiovascular Dynamics. The amount of increase in cardiac output resulting from augmented venous inflow is directly related to the rate of transfusion and the total volume administered. The greater the volume added to the circulation per unit of time the more profound is its effect, and the larger the total volume transfused becomes the greater is the venous pressure and the cardiac output.[18, 22] In individuals with normal hearts no permanent change results from the transfusion of as much as 2 liters *in less than fifteen minutes*, although transient mild cardiac dilatation may occur. In the case of a damaged myocardium cardiac failure may result from the infusion of a much smaller volume.[20] In secondary shock (Chap. 3), where venous pressure and cardiac output are reduced because of lowered blood volume, transfusion returns the cardiovascular dynamics to the normal state. The effect of transfusion on the cardiovascular system then depends upon the state of the myocardium and of the blood volume. The response of the severely anemic individual in whom there may be decreased blood volume, increased cardiac output, and a weakened myocardium has been discussed elsewhere (p. 270).

Changes in Blood Volume. There apparently exists an inherent tendency for the plasma volume to be maintained at a constant level and the total blood volume varies, therefore, as does the

has been made of the concentrated suspension of erythrocytes which has come into rather wide usage in Britain.[24] This preparation of blood is highly recommendable for transfusion in anemia. It is simply blood from which the bulk of the plasma has been removed (Chap. 21). This is accomplished by siphoning the supernatant plasma-preservative mixture from a bottle of sedimented preserved blood. If sterile siphoning apparatus is not readily available the plasma may be successfully decanted from blood stored several days during which time the cell layer will have become firmly packed. This is the ideal preparation for transfusion of anemic or cardiac patients. The number of erythrocytes injected is the same but the total volume is reduced by approximately 50 per cent. One may administer twice as many cells per unit volume by this method as when whole blood is used. The suspension is thick and more viscous than normal blood but will flow through the ordinary transfusion apparatus satisfactorily.

INJURY TO THE RECIPIENT

Local Injury. Hematomata, thrombophlebitis, and cellulitis may follow venipuncture for the administration of blood, occurring in the same manner as when blood is drawn (Chap. 10). These complications of transfusion may be avoided by careful attention to technique.

Air Embolism. Air may enter a transfusion apparatus through faulty joints or defects in the rubber tubing and gain access to the circulation in sufficient quantity to cause symptoms of embolism. A more obvious entry may occur during transfusion under positive pressure. Fatalities resulting from both types of accident have been reported.[25, 26]

Other Reactions. Reactions to transfusion are discussed in Chapter 12, together with the procedures for determining the cause of the accident. To make laboratory tests for hemolytic reactions the samples of blood used in the grouping, typing, and crossmatching before transfusion are necessary. It is therefore advisable to save these samples routinely for twenty-four hours after transfusion. The specimens needed are (1) the recipient's pretransfusion blood, (2) blood from the bottle used in the transfusion, and (3) the recipient's posttransfusion blood. Certain tests are then readily performed. The crossmatch should be checked using the recipient's pretransfusion blood and the sample collected from the administration apparatus. The presence of icterus or hemoglobinemia may be detected by comparison of the serum of the recipient's pretransfusion and posttransfusion blood specimens.

These determinations should be made as soon as possible if the recipient has a chill or exhibits hyperpyrexia during transfusion, particularly in the early part. In addition, examination of the urine for hemoglobin should be made. For this purpose the urine should be collected routinely in the twenty-four hours after transfusion. Lastly the record of the patient's temperature should be examined, even though he have no complaints. If these procedures are followed after each transfusion few reactions will go unnoted and unexplained.

PHYSIOLOGY OF BLOOD TRANSFUSION

The intravenous injection of blood or plasma into a subject with a normal heart produces exactly the opposite effect of hemorrhage. The immediate results are increased venous and right atrial pressure, increased ventricular inflow, and increased cardiac output. There begins concomitantly, however, an adjustment of the circulation and the blood of such nature that both are brought toward the normal state. The exact nature of this adjustment depends largely upon the underlying deficiency for which the transfusion was given.

Changes in Cardiovascular Dynamics. The amount of increase in cardiac output resulting from augmented venous inflow is directly related to the rate of transfusion and the total volume administered. The greater the volume added to the circulation per unit of time the more profound is its effect, and the larger the total volume transfused becomes the greater is the venous pressure and the cardiac output.[18, 22] In individuals with normal hearts no permanent change results from the transfusion of as much as 2 liters in less than fifteen minutes, although transient mild cardiac dilatation may occur. In the case of a damaged myocardium cardiac failure may result from the infusion of a much smaller volume.[20] In secondary shock (Chap. 3), where venous pressure and cardiac output are reduced because of lowered blood volume, transfusion returns the cardiovascular dynamics to the normal state. The effect of transfusion on the cardiovascular system then depends upon the state of the myocardium and of the blood volume. The response of the severely anemic individual in whom there may be decreased blood volume, increased cardiac output, and a weakened myocardium has been discussed elsewhere (p. 270).

Changes in Blood Volume. There apparently exists an inherent tendency for the plasma volume to be maintained at a constant level and the total blood volume varies, therefore, as does the

erythrocyte volume.[27] These conditions hold except in severe anemias in which case there is also a reduction of plasma volume.[23, 27] The changes that take place in blood volume after transfusion depend upon its state before treatment. If there existed a reduced volume of both cells and plasma (e.g., in hemorrhage) transfusion augments both and this increase is permanent. In the case of mild secondary anemia the plasma volume is normal and the erythrocyte volume reduced. Here transfusion initially increases both but the mechanisms protecting the plasma volume quickly bring it to normal. The only permanent increase in blood volume results from the augmented erythrocyte content. In case of overcompensation of erythrocyte deficit or in transfusion of normal subjects, the blood volume is increased by the amount of erythrocytes administered. The plasma and plasma proteins disappear quickly from the circulation. Repeated transfusion of the normal animal produces plethora with a normal plasma volume and an increased cell volume. In each case the plasma volume assumes a normal state and any permanent change in blood volume is equal to the amount of erythrocytes administered.[27–30]

Fate of the Erythrocytes. Ultimately all erythrocytes injected into the recipient's circulation disappear. The rate at which this occurs and the mechanisms responsible are discussed in Chapters 9 and 13. The products of cellular disintegration apparently undergo the same changes as those from indigenous cells so that the iron is salvaged for subsequent hemoglobin formation.

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CHAPTER 12

Transfusion Complications in the Recipient

By Elmer L. DeGowin

It is unfortunate that many able clinicians, who apply the principles of diagnosis to other disorders encountered in medical practice, are content to classify all abnormal manifestations which occur in patients receiving blood as "transfusion reactions." Frequently no attempt is made to differentiate between the types of complications which are known to occur as a result of transfusions. When a patient presents new manifestations of disease during or after the transfusion of blood, the physician is faced with two or more related questions: (1) Is the manifestation due to the primary disease or is it caused by blood transfusion? (2) If the abnormality is caused by transfusion, what type of reaction is it? These questions can only be answered by the clinician thoroughly familiar with the primary disease who performs a physical examination and correlates the findings with the results of laboratory tests. For example, there are many causes for anuria besides hemolyzed blood, so that the occurrence of renal suppression in a patient who receives transfusion cannot be ascribed to the transfusion until evidence of hemolysis is obtained. The appearance of cerebral thrombosis during blood transfusion in an arteriosclerotic patient does not necessarily bear a causal relationship. The patient who has pyelitis may have a chill and fever coincident with the transfusion of blood which contains no pyrogens.

PYROGENIC REACTIONS

This complication is characterized by chills and fever which appear during or a few minutes after transfusion and persist for not

more than a few hours. It is due to the injection into the circulation of pyrogens inadvertently associated with the fluids employed in preserving blood, the fluids used for cleaning apparatus for giving transfusion, or from bacterial growth in the blood itself.

Etiology. In 1923 Seibert[1] demonstrated that certain nonpathogenic bacteria, commonly occurring in river water, can multiply in distilled water and produce substances which cause fever when injected parenterally into animals. These substances were named *pyrogens.* They are carbohydrates which are not destroyed by the heat employed to autoclave rubber tubing and other apparatus for parenteral injection. They pass freely through some filters which hold back bacteria, but they can be absorbed on other filtering substances.[2] Co Tui and his coworkers have shown[3] that pyrogens from *Eberthella typhosa* can be concentrated to such a degree that 0.02 micrograms per kilogram of body weight will cause fever in man when injected intravenously.

The term pyrogen is restricted to bacterial products. Occasionally other substances cause febrile reactions when introduced into the circulation. The finely divided sulfur formerly used in curing rubber tubing is such an agent, but tubing made expressly for parenteral injection apparatus no longer contains this material. The dried denatured plasma proteins in improperly cleaned tubing and apparatus produce violent chills and fever when injected intravenously.

Incidence. Pyrogenic reactions are the most common complication of blood transfusion. The number depends to a great extent on the care with which pyrogens are excluded from the apparatus, fluids, and blood. In 1940 DeGowin and Hardin[4] reported 4.8 per cent reaction of all types in 2423 transfusions of fresh and preserved blood. The incidence of pyrogenic reactions was 2.9 per cent. Transfusions accompanied by chills only were encountered in 0.9 per cent, and those with chills and fever were recorded in 2.0 per cent. There was no significant difference between the incidence of reactions of this type from fresh and from properly preserved blood. This tends to exclude the possibility that pyrogens were formed by bacterial growth after the blood was collected. In 1945 DeGowin[4] reported another series of 5386 transfusions of fresh and preserved blood from the same service in which the same methods were still in use. The incidence of reactions of all types was 3.4 per cent, and reactions with chills and fever, 1.8 per cent. Transfusions with chills but without fever were not included, however.

Clinical Description. During or within an hour after hypodermocylsis, intravenous infusion, or transfusion the patient develops symptoms which may be ascribed to the presence of pyrogens. In the mildest cases there is slight chilliness with no fever. In the more

severe grades there may be violent chills followed by a quick rise in oral temperature to 104° or 105° F. (40° to 40.5° C.). The fever usually persists for less than four hours. During this period the patient may be extremely uncomfortable with prostration, nausea, and vomiting. The skin is flushed, the blood pressure remains unchanged. Complete recovery within twenty-four hours is usual. Pyrogenic reactions seldom occur when the patient is under general anesthesia.

Diagnosis. The association of the symptoms with the parenteral injection of fluids suggests the presence of pyrogens. Pyrogenic reactions must be differentiated from febrile manifestations of the primary disease of the recipient and from hemolytic reactions. The latter are excluded by demonstrating the lack of free hemoglobin and bilirubin in the serum of the recipient in blood collected immediately after the symptoms have occurred. For a discussion on tracing the source of pyrogens consult pages 562 to 565.

Treatment. The injection of the pyrogenic fluid can be discontinued if it is still in progress. Symptomatic treatment may be indicated. *The chill can be stopped by the injection of 10 ml. of calcium* gluconate (10 per cent solution) intravenously and morphine sulfate 0.016 gm. subcutaneously. The early administration of 0.65 gm. of acetylsalicylic acid by mouth is said to abort or modify the symptoms.

Prognosis. While this type of complication may cause great temporary *discomfort, it is not usually dangerous except in patients* who are extremely ill from other disease. Occasionally a recipient with certain infections, such as puerperal sepsis, seems to improve markedly after a pyrogenic reaction in the same manner as if foreign protein had been given.

Prophylaxis. If the injection apparatus is thought to be contaminated *with pyrogens, it may be washed out by running through* and discarding 200 to 300 ml. of sterile pyrogen-free isotonic sodium chloride or dextrose solution before the administration of blood.

Constant vigilance must be employed in the preparation of fluids and apparatus to avoid the introduction of pyrogens. The reader is referred to Chapter 27 for the details of the procedures.

URTICARIAL REACTIONS

Hives and occasionally angioneurotic edema may occur after the transfusion of blood or blood derivatives.

Etiology. Although this complication is usually classified as allergic, in most cases allergy in the usual sense cannot be proved. Extensive questioning reveals no history of allergic manifestations in

most donors or recipients who are concerned in the blood transfusion. We have not been convinced that the collection of blood from fasting donors has materially reduced the incidence of urticarial reactions.

Occasionally an isolated instance is encountered in which an antibody seems to have been transmitted by transfusion. DeGowin and Hardin[4] reported the case of a donor who had had many episodes of angioneurotic edema, the allergenic cause of which could never be ascertained. At least some of the symptoms were initiated by emotional disturbances. He served as a donor in transfusions to ten individuals. Four of the recipients had angioneurotic edema during or soon after receiving his blood. DeGowin observed another instance in which a donor had developed urticaria and arthralgia from taking sulfathiazole. Three weeks later his blood was transfused without evidence of toxicity into a moribund patient who was receiving sulfathiazole. During the transfusion the recipient developed generalized angioneurotic edema with intense erythema. Death occurred a few minutes later but was presumed to be due to the primary disease.

Incidence. Urticaria is the second most common complication in blood transfusion. In a series of 2423 transfusions[4] the incidence was 1.1 per cent; in another series of 5386 transfusions the complication occurred in 0.8 per cent of the cases. These data coincide with the experience of many authors. The occurrence is so constant that Hardin employed the reported incidence of urticaria as an index of the accuracy with which transfusion reactions were reported from various military units in the European Theater of Operations. If the incidence of urticarial reactions was reported as much less than 1 per cent, it could be assumed that the recipients were not being closely observed.

Clinical Description. During or soon after transfusion the hives appear. They are usually localized although occasionally there is a general distribution. The patient suffers the usual discomfort from pruritus. In more severe cases there is angioneurotic edema, most often present on the face and neck. The most serious complication is the development of edema of the glottis, which has not been encountered in our experience. The eruption lasts for only a few hours. It may be accompanied by asthma.

Diagnosis. This is made by inspection of the skin and can hardly be confused with anything else. In the case of asthma the typical breath sounds can be heard in the chest, but the findings must be differentiated from the rhonchi and sibilant rales which occur in edema of the lungs from circulatory overload. Asthmatic breathing should disappear promptly after the administration of epinephrine.

Treatment. Epinephrine hydrochloride, 0.3 ml. of a 1/1000 solution, should be given promptly by hypodermic injection. If the eruption promptly subsides after epinephrine, the transfusion may be resumed. It is probably safer to discontinue the transfusion in the presence of angioneurotic edema.

Prognosis. Prompt recovery without complications is the rule. The occurrence of edema of the glottis is a rare but serious complication.

Prophylaxis. No certain means of prevention is known. Donors should not be accepted who have a history of spontaneous angioneurotic edema or frequent urticaria. Urticaria due to drugs should disqualify the donor if the recipient is likely to receive similar drugs within four weeks after transfusion. Donors with active hay fever or allergic asthma are unacceptable.

CIRCULATORY OVERLOAD

When the blood volume is excessively increased by the addition of fluids or blood, the left side of the heart becomes dilated and sometimes fails acutely. Pulmonary congestion and edema develops. If the condition is not promptly recognized and treated, it is rapidly fatal.

Etiology. The blood volume may be dangerously increased by the injection intravenously of crystalloid solutions, blood, or plasma. In a given situation a smaller amount of whole blood or plasma produces overloading than is the case with crystalloid solutions because the latter leave the circulation faster than whole blood or plasma. Therefore one can reasonably expect that the use of a slow rate of injection for saline or dextrose solutions may prevent dangerous overloading when a faster rate would be disastrous. Overload from blood or plasma is not so dependent on a fast rate of injection because the circulating volume is augmented with colloid solutions and erythrocytes which are more tightly held in the blood stream. A smaller augmentation of the blood volume will produce overloading in a patient with heart failure or in hemorrhagic shock than is the case in other disorders.

Murphy *et al.*[7] studied the effects of the injection of crystalloid solutions intravenously into patients with and without cardiac disease. When 200 ml. of 50 per cent dextrose or 1000 ml. of 10 per cent dextrose was administered the venous pressure was uniformly raised. The same result was obtained when 2000 ml. of isotonic saline solution was injected into patients without cardiac disease. The vital capacity was diminished when patients with cardiac disease were injected, and frank left-sided heart failure occurred at times. For further discussion cons[illegible] 263

Incidence. Data are not available from which to estimate the frequency of circulatory overload as a complication of blood transfusion. When proper methods are employed to prevent incompatibilities, circulatory overload is probably the most common cause of death from blood transfusion. Routine autopsies on patients in a modern hospital reveal frequent instances in which the injudicious augmentation of the blood volume has contributed to death. In addition, where the professional staff is aware of the possibility, many more patients develop the complication but are saved by prompt diagnosis and treatment.

Clinical Description. During transfusion or within an hour afterward the patient suddenly becomes extremely dyspneic, orthopneic, and intensely cyanotic. Large amounts of sanguinous froth may be coughed up. The venous pressure is frequently elevated, as indicated by engorgement of the jugular veins in the erect position. The lungs are filled with sibilant and sonorous rales. The cardiac rhythm may be normal or auricular fibrillation or flutter occurs. The patient may die within a few minutes of the onset of symptoms. If he survives for some hours without treatment, peripheral edema may develop. We have seen massive peripheral edema develop in an infant within a few minutes after overloading.[5]

Diagnosis. The condition must be recognized promptly *at the bedside* by the physical findings. Asthma is the only condition which is likely to cause confusion in diagnosis. The correctness of the interpretation of the findings is confirmed by definite symptomatic improvement and lessening of rales in the chest within five minutes after the application of tourniquets to all four extremities.

Treatment. This is an emergency procedure because a delay of a few minutes may result in death. The first step is the application of tourniquets to the proximal portions of the four extremities. They should be applied snugly enough to prevent venous return but not sufficiently tight to occlude the arterial pulses. Occlusion should not be permitted for over twenty or thirty minutes at a time. If it is necessary to produce stasis for longer periods, one tourniquet should be released at a time, the circulation to that extremity restored, and the tourniquet reapplied before another is released. This method has been shown by Ebert and Stead[3] to pool about 15 per cent of the circulating blood in the extremities, thus removing an appreciable load from the heart. This is equivalent to a temporary removal of 750 ml. of blood from the circulation of a patient with a blood volume of five liters.

The application of tourniquets should be regarded only as a diagnostic test and a first-aid treatment of the condition. As soon

as the symptoms and signs have been alleviated by peripheral stasis, immediate preparations for phlebotomy should be made and the operation performed with the utmost dispatch. A blood collection apparatus similar to that used for donors is satisfactory for the withdrawal of blood. If this is not readily available, a large gauge needle may be inserted into the vein of the patient and the blood permitted to flow into a measuring vessel. We have seen an old-fashioned phlebotomy performed in an emergency with good results. The antecubital fossa was cleaned with alcohol and the point of a narrow-bladed scalpel was inserted into the skin with the cutting edge outward. The vein was transected by thrusting the blade under the vein and cutting up through the skin. The blood flowed off the crook of the elbow into a measuring glass. When the operation was concluded, a tight bandage was applied to the incision so that the bleeding was effectually stopped.

The volume of blood withdrawn should approximate the amount of blood or plasma which has been transfused. If crystalloid solutions have been given in addition, probably only a part of their total volume should be recovered.

The patient should be carefully observed for several hours after definitive treatment has been given because there is a tendency for edema of the lungs to recur if the blood volume has not been sufficiently reduced. If cardiac arrhythmias have occurred as a result of the overload, they should be treated with the appropriate drugs. The physician should be cautioned *not to rely upon drugs for the treatment of the circulatory overload.*

Prognosis. The outcome is hopeful if the condition is promptly recognized and treated adequately according to the principles just outlined. The disorder is frequently fatal if undiagnosed, or when therapy is improperly given.

Prophylaxis. Many cases of overloading of the circulation can be avoided if the physician remembers that success of the injection of fluid or blood intravenously depends on adequate cardiac function. Patients with frank cardiovascular disorders should be given crystalloid solutions slowly and carefully. Transfusion of whole blood or plasma should be administered sparingly and cautiously.

The most careful evaluation of the cardiovascular system, however, will not always yield an accurate prediction of the tolerance of the circulation for augmentation of blood volume. We have seen a young woman with severe mitral stenosis, cardiac failure, and chronic anemia who received several blood transfusions without mishap. A man 75 years of age received ten transfusions of whole blood in a few hours for the treatment of hemorrhagic shock. At some point the circulation was overloaded and he was in unrecog-

nized cardiac failure for 24 hours with the bed in the head-down position. There was edema from the waist to the scalp and the erythrocyte count was 6,500,000 per cu. mm. He recovered completely after the head was raised and 1 liter of blood was withdrawn. These, however, are the exceptions and many persons suddenly succumb to circulatory overload if the condition is not recognized and treated soon.

Protocols of Illustrative Cases.

Case 11.[8] A woman, aged 30 years, had bacteremia caused by *Streptococcus hemolyticus* and thrombosis of a large cavernous hemangioma involving the entire right arm. The heart was normal in size and rhythm. A mixture of 500 ml. of citrated blood and 200 ml. of isotonic saline solution was transfused in seventy minutes. One hour later extreme cyanosis and dyspnea occurred and the patient complained of indefinite pains in the legs and thighs. Coarse rales were heard in the left lung. Morphine sulfate, epinephrine hydrochloride, and atropine sulfate were administered with temporary improvement, but death occurred eight hours later.

In addition to the findings of septicemia, the autopsy revealed pulmonary congestion and edema of the lungs. The heart was not dilated post mortem.

Case 12.[8] A woman, 43 years of age, entered the hospital with chronic glomerular nephritis and secondary anemia. Previously she had had hypertension, but the blood pressure at this examination measured 140/70. The heart was moderately enlarged and there was slight pitting edema of the ankles The urine contained albumin and a few leukocytes. A transfusion was begun, consisting of 450 ml. of citrated blood and 150 ml. of isotonic saline solution When 200 ml. of the blood mixture had been injected in forty-five minutes, severe cyanosis and dyspnea occurred and the transfusion was promptly discontinued. Epinephrine hydrochloride and morphine sulfate were given subcutaneously and aminophylline was injected in the vein. The patient died thirty minutes after the symptoms appeared.

At autopsy there was intense pulmonary congestion and edema. The heart was somewhat hypertrophied and the chambers were moderately dilated. The kidneys showed evidence of chronic diffuse nephritis The postmortem blood was examined chemically. The blood urea nitrogen was 99.4 mg. per 100 ml. and the creatinine 11 8 mg. per 100 ml. There was no free hemoglobin in the blood serum and no agglutinates of erythrocytes were found in the blood stream.

Unreported Case. A man, 45 years of age, had had a thyroidectomy on the previous day. The circulatory system was considered normal from physical examination. An intravenous infusion of 800 ml. of 5 per cent dextrose solution and the same amount of 0 9 per cent saline solution was given at the rate of approximately 20 ml. per minute. About thirty minutes after the completion of the infusion he suddenly became intensely cyanotic and orthopneic. The lungs were filled with coarse rales. The

cardiac rhythm was regular but the rate was 180 beats per minute. Tourniquets were applied to the four extremities with disappearance of the rales, cyanosis and dyspnea within five minutes. A phlebotomy was performed and 500 ml. of blood was withdrawn. The tourniquets were released with no recurrence of symptoms. But the tachycardia persisted and an electrocardiogram showed auricular flutter. Quinidine lactate (0.65 gm.) was administered intravenously and the rhythm converted to normal within twenty minutes. There were no further circulatory symptoms. Although the circulatory overload in this case was caused by crystalloid solutions and not by blood transfusion, the same principles of treatment apply in both instances.

HEMOLYTIC REACTIONS

When excessive amounts of hemolyzed blood are present in the circulation profound changes in the physiologic processes occur which are classified as hemolytic reactions. Perhaps more attention has been devoted to this type of transfusion complication than to any other, but there are still gaps in our knowledge.

Etiology. There is ample clinical evidence to prove that some element or elements from ruptured red cells are toxic when they occur in the circulation in sufficient amounts. The cellular debris from erythrocytes have been considered harmful, but Heidelberger[10] injected large quantities of stromata from human red cells into the veins of human beings without perceptible reaction.

The potassium content of human erythrocytes is normally between seventeen and twenty times as high as that of the plasma. When the serum potassium of dogs attains a concentration of 5 to 11 millimols per liter, Winkler, Hoff, and Smith[11] showed that cardiac arrest occurs. The question was raised by Scudder, Drew, Corcoran, and Bull[12] as to whether the amount of potassium transfused in the plasma of preserved blood might not be toxic. DeGowin, Hardin, and Harris[13] transfused 500 ml. amounts of preserved blood into human recipients. The blood had been stored for a sufficient period so that approximately three quarters of the potassium had diffused from the erythrocytes into the plasma. No toxic manifestations or electrocardiographic changes were produced in the recipients by this procedure. It seems likely from these experiments and much clinical experience that potassium is not an important factor in the phenomena associated with hemolytic reactions.

Most workers concede that free hemoglobin released by the rupture of red cells is probably the toxic substance which causes the severe complications in hemolytic reactions.

Removal of Free Hemoglobin from the Circulation. Several groups of workers have studied the rate of disappearance of free hemoglobin

from the blood plasma of the human subject. The most extensive observations were reported by Gilligan, Altschule, and Katersky[14] who injected ten normal persons intravenously with from 1.3 to 16.4 gm. of hemoglobin in solution. The plasma hemoglobin levels rapidly attained a maximum and then fell to normal in from six to twenty-four hours after injection. The higher the maximal concentration, the greater the rate of fall. Whenever the plasma hemoglobin attained a concentration over 135 mg. per 100 ml., free hemoglobin and some albumin appeared in the urine. The hemoglobinuria persisted until the plasma level of the pigment decreased to 30 to 50 mg. per 100 ml. If there was preexisting albuminuria, the renal threshold was much lower and hemoglobinuria occurred when the plasma level of the pigment reached 40 to 50 mg. per 100 ml. The albuminuria associated with the hemoglobin in the urine usually persisted for an hour or so after the pigment had disappeared from the urine. From experiments in dogs Lichty *et al.*[15] found that the glomerular threshold for hemoglobin is quite low but the pigment is reabsorbed by the tubular epithelium until the latter becomes temporarily blocked, thus accounting for the hemoglobinuria which occurs with the low plasma concentration of pigment.

Ottenberg and Fox[16] found a more variable renal threshold for hemoglobin in twenty human subjects to which intravenous injections were given. Hemoglobinuria appeared in four of eight persons with plasma hemoglobin levels from 110 and 150 mg. per 100 ml., whereas it appeared in but nine of fifteen cases with plasma hemoglobin levels from 175 to 300 mg. per 100 ml.

Only from 12 to 30 per cent of the hemoglobin which disappears from the plasma is excreted in the urine.[15] Some is slowly converted to bilirubin and is taken up by the reticuloendothelial system. After injections of hemoglobin intravenously the plasma bilirubin rises from six to eight hours.[14] When 16.4 gm. of hemoglobin was injected, the plasma bilirubin attained a value of 1.1 mg. per 100 ml. There must be great individual variability in the rate of conversion of hemoglobin to bilirubin because DeGowin[6] observed a woman (Case 38-10586) whose plasma contained 6.1 mg. of bilirubin per 100 ml. immediately after a hemolytic transfusion reaction, but there was no free hemoglobin in the plasma. The urine contained bilirubin for twenty-four hours afterward but no hemoglobin was excreted. A small amount of hemoglobin circulating in the plasma for over six hours is converted into a compound which Fairley[17] has described and termed *methemalbumin*. This persists in the plasma for over twenty-four hours and can be identified by spectroscopy.

Amount of Free Hemoglobin Causing Symptoms. There is fairly good agreement as to the amount of free hemoglobin in the circulation which can cause symptoms. In the studies previously cited[14] no subjects receiving hemoglobin suffered symptoms from doses less than 10 gm. A person who received 10 gm. developed severe abdominal cramps, vomiting, and visible peristalsis. These manifestations were relieved by the intravenous injection of calcium chloride. When 16.4 gm. of hemoglobin was injected into a subject a moderate chill was followed by abdominal cramps, pains in the legs, and a slight fever lasting fifteen hours. O'Shaughnessy *et al.*[18] described chills, fever, pains in the back and loins, and a sensation of constriction in the chest from solutions containing between 10 and 50 gm. of hemoglobin. Cannan and Redish[19] injected solutions of crystallized human hemoglobin intravenously into normal subjects. They observed no symptoms until the dose was increased to 0.64 gm. per kilogram of body weight (44.8 gm. for a person weighing 70 kilograms). The larger doses resulted in epigastric discomfort, nausea, vomiting, diarrhea, and slight fever.

Renal Ischemia. Mason and Mann[20] observed a transient decrease in the volume of the kidneys of the dog and constriction of the frog's glomeruli during the injection of hemoglobin solutions intravenously. Hesse *et al.*[21] made extensive experiments on dogs to which injections of hemolyzed blood were given intravenously. The renal vessels were constricted, which caused a transient diminution in the volume of the kidneys. Renal ischemia certainly must be considered as a possible factor in the development of transfusion anuria.

Renal Blockage. Baker and Dodds[22a] produced obstruction of the lumina of the renal tubules in rabbits. The hemoglobin precipitated in the tubules when the urine was acid but this did not occur in the presence of an alkaline urine. They showed that the precipitation of hemoglobin in an acid medium was augmented when the concentration of sodium chloride exceeded 1 per cent.

DeGowin *et al.*[22b,c] produced fatal renal insufficiency in dogs by inducing hemoglobinuria when the urine was made acid by feeding beef muscle and ammonium chloride. Renal damage did not occur with hemoglobinuria in the presence of an alkaline urine when the diet consisted mostly of carbohydrate and fat. The histologic examination of the kidneys of dogs dying with azotemia revealed evidence of obstruction of the tubular lumina in the region of Henle's loops with casts of precipitated hemoglobin and crystals of pigment. But there were places remote from the obstruction in which the tubular epithelium was necrotic. The appearance of the dog kidneys was compared with that of nine human beings who had died of renal insufficiency after intravascular hemolysis. In the

human kidneys evidence of both the obstructive and the nephrotoxic processes could be found, but in most the obstruction did not seem to be sufficiently extensive to cause death. It was believed that the obstructive process could probably be prevented by alkalinization of the urine. The authors concluded that the chief lesions were tubular degeneration and interstitial edema, the cause of which was unknown.

More recently, the importance of the obstructive mechanism and its relation to urinary acidity has been questioned. DeNavesquez[23] found that the kidneys of rabbits having hemoglobinuria with an acid urine contained less iron than those with an alkaline urine. He concluded that a low pH of the urine was not a factor in the production of renal damage. An attack of hemoglobinuria was induced in a patient by immersion of the arm in ice water when the urine was acid, but no evidence of renal damage resulted. It was estimated that only about 13 gm. of free hemoglobin was released by this maneuver. This is probably an insignificant dose as judged by clinical observations and the studies previously cited.

Bing[24] injected solutions of oxyhemoglobin and methemoglobin into dogs which had been made acidotic. He concluded that oxyhemoglobin was innocuous but methemoglobin produced renal damage. Van Slyke *et al.*,[25] however, demonstrated that when methemoglobin was injected into dogs it was promptly converted to oxyhemoglobin by the glycolytic system in the erythrocytes. They concluded that oxyhemoglobin was sometimes toxic to the kidneys.

Flink[26] published an excellent review of the subject of hemoglobin toxicity and reported the results of his studies on dogs. Hemoglobinuria was produced by the intravenous injection of solutions of the pigment and serial biopsies of the kidneys were made with a Silverman needle. Approximately equal amounts of renal damage were observed in the presence of acid and alkaline urine. Lalich[47] concluded from experiments on rabbits that dehydration caused hemoglobin precipitation in the kidneys when hemoglobinuria was present.

Some of the discrepancies seem to have been reconciled by the experiments of Yuile, Gold, and Hinds[27] on dogs. Hemoglobinuria was relatively harmless in the presence of a normal kidney. On the other hand, when hemoglobinuria occurred after the kidney had been previously damaged by a short period of ischemia, there was some precipitation of hemoglobin in the renal tubules. The process was much more extensive when the urine had previously been rendered acid in reaction. It is possible that the necessary renal ischemia occurs clinically during the period of primary shock.

Primary Shock. The studies of Hesse *et al.*[21] demonstrated that free hemoglobin in the plasma causes a considerable drop in the systemic arterial tension. It seems probable that this can produce renal ischemia and serve as an initial factor in renal damage. DeGowin and Hardin[6] observed the case of a woman belonging to group O who was inadvertently given a transfusion of group A blood. After 100 ml. of blood had been injected a violent chill occurred with dyspnea and cyanosis. Although the transfusion was promptly stopped extreme prostration occurred with the arterial tension at shock level. The blood plasma was red with free hemoglobin. In spite of a transfusion of 500 ml. of compatible blood, the patient died in shock about six hours after the reaction. Her blood contained a potent anti-A hemolysin but no agglutinates were found. Since then observations have been made on two patients with hemolytic transfusion reactions. Immediately after hemolysis occurred systolic pressure measurements were found to be less than 80 mm. of mercury. The existence of shock was not suspected by inspection of the patients because there was no pallor, and prostration was not a prominent feature. In one instance (Case 44-6538[5]) the hypotension was present for at least twenty-four hours after hemolysis occurred. Because of the lack of hemorrhage and the presence of vasodilatation, this syndrome must be regarded as primary shock.

Causes of Hemolysis. The causes of hemolysis in blood transfusion reactions are conveniently classified as extravascular and intravascular.

Extravascular Hemolysis. This occurs from improper handling of the donor's blood before it is injected into the recipient's vein.

1. *Freezing.* The erythrocytes rupture when the contained water has been frozen. The freezing point of the cell contents is about −0.56° C. although the plasma proteins permit supercooling to as low as −10° C. under certain temporary conditions.

2. *Heating.* The injudicious application of heat to a flask of blood may result in hemolysis. One case has been reported[28] in which overheated blood caused urinary suppression.

3. *Improper Storage.* When blood has been stored for some days at room temperature, or for longer periods at 2° to 10° C., hemolysis occurs (p. 305). Transfusion of blood with excessive spontaneous hemolysis from improper storage will cause reactions.

4. *Addition of Hypertonic or Hypotonic Solutions.* We have observed instances in which distilled water was added to blood mixtures before transfusion and hemolysis, of course, resulted. The admixture of blood with hypertonic solutions, such as 50 per cent dextrose, will also cause hemolysis.

5. *Diffusion of Dextrose.* If blood properly preserved in one of the standard dextrose-citrate solutions is taken from the refrigerator and permitted to stand for several hours at room temperature, hemolysis may occur. This is caused by the diffusion of an added increment of sugar into the erythrocytes as the temperature slowly rises, when the cell contents have already been altered by several days of normal storage.[29] It occurs more frequently when the preservative mixture produces a relatively large dilution of the plasma proteins. By the same mechanism, hemolysis may occur when a large volume of 5 per cent dextrose at room temperature is added to a flask of cold blood in a dextrose-citrate mixture. Occasionally the mistake is made of adding Ringer's solution to blood containing sodium citrate. The calcium in the Ringer's solution causes clotting of the blood. This merely results in frustration but is not dangerous to the patient because the blood cannot be injected. The addition of calcium gluconate to citrated blood has resulted in intravascular thrombosis.

Intravascular Hemolysis. This refers to the rupture of the erythrocytes in the circulation of the recipient. They may be either the donor's or the recipient's red cells. Since all red cells are sooner or later destroyed *in vivo*, the definition pertaining to transfusion reactions must further specify that the erythrocytes are destroyed in sufficient quantities within such a short time as to produce a dangerous concentration of hemoglobin in the plasma of the recipient.

1. *Increased Osmotic Fragility of Donor's Cells.* During the storage of blood the contents of the erythrocytes may become relatively hypotonic with respect to normal plasma (p. 321). These cells may be transfused intact but contact with the plasma of the recipient causes rupture. This situation usually occurs from a combination of events in which the cell contents become hypotonic during storage and an increased amount of dextrose diffuses into them.

2. *Incompatible Hemolysins in Recipient's Plasma.* When the recipient's plasma contains anti-A or anti-B isohemolysins, as well as the corresponding agglutinins, transfused incompatible cells are first clumped and then hemolyzed. The hemolysins are activated by the complement in the recipient's plasma.

3. *Incompatible Isohemagglutinins in the Recipient's Plasma.* In many transfusions where incompatible blood is given, no hemolysins can be demonstrated. This is true in about 70 per cent of the cases in which the incompatible agglutinins are anti-A or anti-B, and probably in all cases in which anti-Rh or anti-Hr antibodies are involved. There is no doubt that the agglutinates which are formed under these conditions are somehow hemolyzed. A plausible explanation of the mechanism of hemolysis seems to have been found

in the observations of Shu, Castle, and Fleming[30] that the erythrocytes agglutinated by the action of antibodies are more readily ruptured by mechanical trauma than are the unagglutinated red cells. This suggests the possibility that the agglutinates may be hemolyzed by the trauma of the circulation.

4. *Transfusion of Incompatible Antibodies in Donor's Plasma.* Usually when incompatible antibodies are present in the donor's plasma they are sufficiently diluted in the plasma of the recipient and fixed by the group-specific substance in the body cells of the recipient so that the action on the recipient's blood cells is neglible. Occasionally, however, when the donor's antibodies are unusually potent, the recipient's cells may be clumped and destroyed. This has been most intensively studied in the problem of the universal donor (p. 245).

5. *Hemolysis Coincident With Transfusion but from Other Causes.* The authors have observed instances in which intravascular hemolysis was noted after transfusion but the cause was found to be the injection intravenously of distilled water as a preliminary to transfusion. A number of cases have been observed by the authors in which intravascular hemolysis was found after transurethral resection of the prostate gland. Transfusion had been given in the treatment of hemorrhagic shock. It was found that the routine procedure of instilling sterile tap water into the bladder under pressure to stop hemorrhage actually resulted in forcing the water into the circulation. This has been reported by others.[31]

Incidence. It is doubtful whether any large series of blood transfusions, has ever been given without hemolytic complications, some of which have been fatal. The number of such accidents depends upon the care employed in handling the donors' blood and the excellence of the laboratory technique used in grouping, typing, and crossmatching. Although measures to insure compatibility in the A-B-O and Rh-Hr systems will prevent serious intravascular hemolysis most of the time, these will not completely eliminate the complication. Probably one to five hemolytic reactions per 1000 transfusions are inevitably caused by human error.

Clinical Description. Some transfusions associated with hemolysis are not accompanied by symptoms of any sort. In general, these are cases in which hemolysis is minimal, although this is not always true.

Precursory Stage. The interval between the beginning of the transfusion and the appearance of symptoms varies somewhat and probably depends on the dose of hemoglobin released or injected, as the case may be. When potent incompatible antibodies cause hemolysis, symptoms may occur when as little as 50 ml. of blood

has been injected. Hemolysis caused by the anti-Rh agglutinins seems to proceed at a slower rate because the symptoms sometimes appear from one to two hours after the transfusion is concluded. Frequently the injection of blood which has been hemolyzed extravascularly does not cause symptoms. This is probably because the concentration of hemoglobin which can be attained by intravenous injection of hemolyzed blood is less at any one time than that which is the result of a large amount of intact erythrocytes disintegrating within the circulation.

Early Symptoms. The patient experiences severe pain in the loins, the lumbar region, and down the legs. There is a sense of constriction substernally. This is quickly followed by violent chills and fever, with the temperature attaining values as high as 104° F. (40° C.). The skin is frequently flushed and the respiratory movements are rapid and labored. There may be great prostration for a few hours. The systolic blood pressure may be depressed to 60 mm. of mercury or lower. The hypotension may be present for only a few minutes or for over twenty-four hours. The patient rarely dies during the stage of primary shock although this has been observed. Many or most of the early symptoms are masked if the transfusion is given while the recipient is under general anesthesia.

Jaundice. Seldom sooner than twelve hours after transfusion in which hemolysis has occurred the patient may become icteric. The jaundice persists for several days but is usually deepest on the first day and subsides progressively thereafter. Icterus does not occur in all patients in whom hemolysis occurs. Frequently the van den Bergh reaction on the serum is "direct" soon after the appearance of clinical icterus. The reaction later may change to "biphasic" or "indirect." No satisfactory explanation has been advanced for this observation.

Renal Insufficiency. Many recipients who develop the early symptoms associated with hemoglobinemia continue to secrete urine satisfactorily and appear none the worse for the experience within twenty-four hours after transfusion. Hemoglobinuria occurs within a few minutes after the hemoglobin level in the blood plasma has attained the renal threshold and continues for from a few to thirty-six hours. Transient albuminuria usually accompanies the hemoglobinuria. The volume of the urine may actually be increased during the stage of hemoglobinuria.

A minority of the recipients with hemolysis from transfusion develop symptoms and signs of renal insufficiency. Writers are generally agreed that serious renal damage has not been observed in adults with hemolysis of less than 200 ml. of blood. The early symptoms are followed by progressive lassitude, prostration, nausea,

and vomiting. Frequently the patient becomes irrational. Convulsions may occur. Finally coma supervenes and death occurs between the fourth and fifteenth day after transfusion. The daily urine volume is between 800 ml. and zero. Inexperienced clinicians are frequently deceived into thinking that a urinary output of 500 ml. per day excludes the possibility of renal insufficiency. The arterial blood pressure usually remains normal although Daniels *et al.*[32] reported several cases in which hypertensive levels were attained. Any time during the period of oliguria or anuria spontaneous diuresis may occur and recovery follow. This is accompanied by a gradual disappearance of the signs of azotemia. On each successive day of renal insufficiency the blood urea and creatinine increase; the greatest augmentation in the concentration is usually observed during the first twenty-four hours.

Diagnosis of Hemolytic Reactions. The early symptoms accompanying the introduction of free hemoglobin into the circulation are not sufficiently distinctive to differentiate the condition from severe pyrogenic reactions. The only practical direct proof is obtained by the demonstration of hemoglobinemia or bilirubinemia in the recipient soon after the transfusion has been given. This requires: (*a*) The pretransfusion specimen of blood collected from the recipient for grouping and crossmatching. (*b*) A posttransfusion specimen of the recipient's blood, collected with a dry, chemically clean syringe and placed in a test tube similarly prepared. Both specimens are centrifuged and the color of the plasma or serum is compared. If the posttransfusion plasma or serum is red, this is sufficient evidence that it contains over 10 mg. of free hemoglobin per 100 ml. Hemolysis of some degree may be assumed if the posttransfusion specimen of serum or plasma is a deeper yellow than the control. (*c*) The presence of hemoglobin, without erythrocytes, in the posttransfusion urine specimen is positive evidence of hemolysis, although its absence does not preclude the possibility of a concentration of hemoglobin in the blood plasma below the renal threshold.

Diagnosis of the Cause of Hemolysis. After demonstrating the fact of hemolysis the mechanism should be investigated. For a complete study the following are required: (*a*) The pretransfusion specimen of the recipient's blood, clotted, citrated, or oxalated. (*b*) The posttransfusion specimen of the recipient's blood. (*c*) A specimen of the donor's blood, preferably in the flask from which the transfusion was given. (*d*) Flasks containing residues of crystalloid solutions or other fluids given intravenously to the recipient before or after the blood transfusion. The following procedures are recommended in approximately the order given:

1. *Check the Labels on the Blood Flasks.* Ascertain whether the flask of blood was given to the recipient for whom it was intended. Each container should bear a label giving the name of the intended recipient. Instances have been encountered in which two transfusions being given in the same unit of the hospital have resulted in giving the wrongs flasks of blood to the recipients, although the labels were clearly indicative.

2. *Rapid Osmotic Fragility Test on Donor's Blood.* Perform the test (p. 198) on the residuum of donor's blood in the flask. If there is excessive free hemoglobin in the plasma of the control tube, hemolysis has occurred extravascularly. If there is hemolysis only in the tube to which isotonic saline has been added, the erythrocytes probably ruptured in the plasma of the recipient.

3. *Grouping of the Donor's and Recipient's Cells.* Repeat the blood grouping on the cells of the donor and recipient. The specimen of donor's blood should be obtained from the residuum in the flask, if available, to detect possible error in labeling the flask. The posttransfusion specimen of the recipient should be used for grouping.

4. *Crossmatching in the A-B-O System.* Crossmatching with the centrifuge technique (p. 183) should be performed if the blood groups of the donor and recipient are likely to be compatible. Examine the tubes quickly to obviate the action of hemolysins on possible agglutinates. Use the pretransfusion specimen of the recipient and the donor's blood from the flask.

5. *Crossmatching in the Rh-Hr System.* Use the serum from the pretransfusion specimen of the recipient and the donor's cells from the transfusion flask. Suspend the donor's cells in albumin solution or serum from AB blood; add no saline solution. Employ the test tube method with incubation for sixty minutes (p. 186). This should demonstrate, in addition to incompatibility in the Rh-Hr system, significantly potent irregular agglutinins such as anti-A_1 and anti-O (anti-A_2), and anti-P.

6. *Typing for Rh-Hr.* If incompatibility is shown in step 5, determine the subtype of Rh-Hr to which the cells of the donor and recipient belong. If no incompatibility is apparent in the results, type for the M-N agglutinogens.

7. *Nontransfusion Causes.* (*a*) Check the label of the saline solution given with the blood transfusion. Take a sample in a test tube and add a few drops of silver nitrate to obtain a white precipitate of silver chloride, demonstrating the presence of chloride in the solution. Add 1 ml. of the donor's blood to a test tube containing about 5 ml. of the sodium chloride solution. Mix and centrifuge to determine if the cells are hemolyzed in the saline

solution. (*b*) Determine whether dextrose or other solutions at room temperature were added to the cold preserved blood before transfusion. (*c*) Ascertain whether the recipient has a hemolytic anemia such as nocturnal hemoglobinuria or cold hemoglobinuria. (*d*) Determine the titer of cold agglutinins in the blood of the recipient (p. 193). (*e*) Perform the Donath-Landsteiner test on the donor's blood. (*f*) Ascertain whether the recipient has recently had a transurethral prostatic resection with water instilled in the bladder.

Treatment of the Primary Shock. The renal ischemia accompanying the hypotension might predispose to tubular damage from hemoglobin and measures should therefore be taken to augment the arterial pressure. Subcutaneous injections of either epinephrine hydrochloride or ephedrine sulfate will maintain normal levels of blood pressure. A trial of amphetamine is suggested. None of these drugs is recommended without reservation, however, because it is not known how much they diminish the circulation of the kidney.

Treatment of the Renal Insufficiency. The definitive treatment of transfusion anuria is unknown. Therapy in this condition is difficult to evaluate because the observed instances are rare, the condition is desperate so that many procedures are tried on the same patient, and spectacular spontaneous recovery is known to occur.

Discontinuance of the Transfusion. The administration of blood should be promptly stopped as soon as symptoms of a reaction occur so that the presence or absence of hemoglobinemia may be determined. The prognosis for recovery may be dependent upon the amount of free hemoglobin in the circulation.

Fluid Replacement. Some observers believe that the parenteral injection of crystalloid solutions should be employed sparingly and only enough should be given to replace that lost in the vomitus, the feces, the sweat, and the insensible perspiration. There is a natural tendency to give too much fluid in an effort to encourage diuresis.

Diathermy and Irradiation of the Kidney Regions. These procedures have failed in our hands.

Transfusion of Compatible Blood. The procedure was first advocated by Hesse *et al.*[21] and they reported cases in which the therapy seemed to be successful. It failed in the only case in which we tried it in the treatment of primary shock (p. 278).

Decapsulation of the Kidneys. The operation was first suggested for the treatment of transfusion anuria by Bancroft.[33] Successes

and failures have been reported by various writers. We have observed failure in the two cases in which it has been tried. Histologic examination of the kidneys, however, sometimes reveals considerable interstitial edema which might not cause pressure on the renal tubules if decapsulation permitted the kidney mass to expand.

Spinal Anesthesia. Procaine hydrochloride injected directly into the spinal fluid was first suggested by Hayes and Paramore.[34] The method has failed us in the several instances in which it has been tried. Another patient[8] recovered from anuria although there remained some doubt as to whether the treatment was responsible.

Procaine Hydrochloride Intravenously. The slow injection of 1 gm. of procaine hydrochloride in 500 ml. of isotonic saline solution intravenously was suggested by Berne.[35] It has proved successful in anuria from other causes and deserves further trial.

Alkalinization of the Urine. In view of the uncertainty as to the mechanism of transfusion anuria, the effect of this procedure cannot be evaluated. There is sufficient experimental evidence to suggest that less renal damage from hemoglobin occurs when the urine is alkaline in reaction (see discussion of etiology) so that the procedure is justified as long as there is no definitive treatment. The amount of base which must be given to produce an alkaline urine varies depending on the acid-base balance of the patient when treatment is given. Sodium bicarbonate or sodium citrate may be given by mouth in amounts of 8 to 10 gm. The intravenous injection of 1000 ml. of M/6 sodium r-lactate solution will usually produce an alkaline urine in an adult. Alkalinization of the urine after anuria has occurred may be too late to prevent damage. The prophylactic administration of alkalis would probably be more effective. In a careful transfusion service the occurrence of hemolytic reactions is so rare as to raise the question of whether the delay of transfusion necessitated by preliminary alkalinization might not result in a greater mortality from hemorrhagic shock.

Peritoneal Irrigation. Frank, Seligman, and Fine[36] introduced the procedure of peritoneal irrigation in the treatment of anuria from various causes. The peritoneum is temporarily substituted for the glomeruli as a membrane through which the products of nitrogen metabolism may be dialyzed until the kidneys recover their function. Two tubes are inserted into the peritoneal cavity through widely separated incisions. Through one opening sterile Tyrode's solution, containing heparin and penicillin, is run into the peritoneal cavity at a rate of 40 to 60 ml. per minute, for a total of

30 to 35 liters in twenty-four hours. A metal sump is placed in the other incision and the fluid is aspirated through this. From 15 to 20 gm. of urea can be cleared in twenty-four hours by this method. The irrigation can be carried on for several weeks. In some cases the azotemia has been successfully ameliorated until renal function resumes spontaneously. There are many technical difficulties to be overcome. A low grade peritonitis may develop. Severe generalized edema frequently ensues. The outlet and the inlet tubes often become plugged with fibrin which seriously impairs or stops the irrigation in spite of the added heparin.

Prognosis. No exact figures can be stated for the mortality from hemolysis as a result of blood transfusion. Most patients survive small amounts of free hemoglobin in the blood stream. Few die of primary shock. The majority with hemoglobinuria do not develop renal insufficiency. Of the recipients in whom anuria or oliguria develop, probably 50 to 75 per cent die with the present methods of treatment. When transfusions are carefully checked for compatibility, circulatory overload probably accounts for more deaths than hemolysis.

Prophylaxis. It should be evident that the prevention of hemolytic reactions is much more practicable than the treatment. The prophylaxis includes (*a*) care in the storage and handling of donor's blood, (*b*) meticulous attention to labeling of flasks and keeping of records, (*c*) competent and complete tests for compatibility before transfusion, (*d*) prompt termination of transfusion when symptoms of complications first occur, (*e*) thorough investigation of the cause of hemolytic reactions, (*f*) treatment of the recipient in the phase of primary shock, (*g*) probably alkalinization of the urine as soon as hemolysis is suspected.

Pathology of Transfusion Anuria. There is general agreement on the pathologic findings in patients dying of renal insufficiency as a result of hemolysis. The renal lesions have been variously called *transfusion nephrosis*, *lower nephron nephrosis*,[48] or *hemoglobinuric nephrosis*.[49] Frequently there is some necrosis of the cells in the central zones of the liver lobules. If sufficient hemoglobin has been released, hemosiderin granules are deposited in the Kupffer cells of the liver and the epithelial cells of the proximal convoluted tubules of the kidneys. DeGowin *et al.*[226] have shown that this deposition of pigment is not harmful but occurs regardless of whether renal damage is present. When renal insufficiency has resulted in death, the kidneys are slightly enlarged and congested. In fresh sagittal sections the cut surface sometimes presents brownish radial streaks in the medulla. Microscopically the

kidneys are seen to contain interstitial edema. There is some polymorphonuclear leukocytic infiltration in the interstices. The glomeruli appear normal. The lumina of the convoluted tubules are dilated. Casts of a brown pigment are found in variable numbers in the lumina of Henle's loops and the collecting tubules. The pigment does not stain blue with potassium ferrocyanide, but is presumed to be a derivative of hemoglobin. The casts are usually not sufficiently numerous to obstruct enough tubules to impair renal function.[224] The tubular epithelium adjacent to the casts is frequently necrotic, but there are many other areas in the convoluted tubules in which the presence of necrosis is unassociated with casts. The tubular lumina usually contain pigment, leukocytes, and cellular debris. The tubules are sometimes lined with low cuboidal epithelium, which suggests that there has been previous sloughing or superficial degeneration. Occasionally, when the patient has lived for several days after hemolysis, the cells of the tubular epithelium contain mitotic figures which indicate regenerative processes. This suggests the possibility of recovery if the renal function could be temporarily supplemented by peritoneal irrigation.

A similar pathologic picture is encountered in death from hemolysis unassociated with transfusion, such as quinine hemolysis, hemorrhagic shock, crush syndrome, and blackwater fever. The extensive material at the Army Medical Museum has recently been described by Lucké.[45] Mallory[47] has studied the renal lesions in hemorrhagic shock.

Protocols of Illustrative Cases.

Case 1.[8] A woman, 53 years old, belonging to group O, was given a transfusion with a suspension of erythrocytes of the same group. Immediately after transfusion she complained of severe pain in the neck, thighs, and abdomen. There was a chill and the rectal temperature rose to 103.2° F. (39.5° C.). Jaundice appeared in seven hours. The urine was diminished in volume and contained free hemoglobin and brown granular casts. Vomiting was frequent. Drowsiness and generalized edema developed. Death occurred nine days after transfusion. The treatment consisted of intravenous injections of isotonic saline and hypertonic dextrose solutions, diathermy to the kidney regions, and irrigation of the renal pelves with warm water.

The preliminary crossmatching had demonstrated no incompatibility. After the reaction occurred, further tests showed that the patient's serum hemolyzed the donor's cells. At that time the Rh factor was unknown and compatibility tests were made without incubation. The chemical findings are summarized in Table XIX.

TABLE XIX

Laboratory Findings in Case 1

Days After Transfusion	Serum Van den Bergh Mg. Bilirubin per 100 ml	Blood Urea Nitrogen Mg. per 100 ml.	Blood Creatinine Mg. per 100 ml.	CO_2 Combining Power of Plasma Volumes per 100 ml.	Plasma Chlorides Mg. per 100 ml.
1	17.4 direct				
2	0.9 direct	79.4	4.0	35.0	
3	0.9 direct	86.8	7.7	34.1	536
4		91.9	10.0		
6		94.5	12.0	26.8	555
7		100.0	11.5		
8		102 0	12.0	24.0	555
9 DIED					

Case 2.[8] A man, 65 years old, belonging to group O, was given blood purporting to belong to the same group. The preliminary crossmatching showed no incompatibility. After 75 ml. of blood had been injected, the patient complained of a cramp in the muscles of the thigh. This symptom was disregarded and a total of 500 ml. of blood was transfused. One hour after completion of the transfusion the patient became nauseated and vomited. A slight chill was followed by a rise in the rectal temperature to 100.4° F. (38° C.). Oliguria occurred and persisted during the rest of his life. Vomiting continued and the patient became stuporous and died in coma ten days after the transfusion. No clinical jaundice was apparent.

The treatment consisted of the injection of isotonic saline solution intravenously and by hypodermocylsis, hypertonic dextrose solution intravenously, and irrigation of the renal pelves with warm water. The autopsy revealed necrosis of the central portions of the hepatic lobules and necrosis of the tubular epithelium of the kidneys. The lumina of a few tubules were filled with debris and hemoglobin pigment

After hemolysis was diagnosed, crossmatching again demonstrated no incompatibility between the donor's and recipient's blood. Three years later the blood of the donor was tested with more potent antisera and found to belong to group A. The chemical findings are found in Table XX.

Case 6.[8] A woman, aged 46 years, belonging to group A, was transfused with 500 ml. of blood of homologous group from her husband; the preliminary crossmatching had shown no incompatibility. Thirty minutes after transfusion she had chills and the oral temperature rose to 104.2° F. (40.1° C.). There was considerable vomiting and an increase in the vaginal bleeding for which she had entered the hospital. Twenty-four hours later jaundice and oliguria were noted The significance of the diminished urinary volume was difficult to evaluate because of the excessive outside temperature which had caused profuse sweating. Spinal anesthesia was administered on the second day with no change in the urinary output. Spontaneous diuresis occurred on the fourth day and the symptoms rapidly improved.

TABLE XX

Laboratory Findings in Case 2

Days After Transfusion	Serum Van den Bergh Mg. per 100 ml.	Blood Urea Nitrogen Mg. per 100 ml.	Blood Creatinine Mg. per 100 ml.	CO_2 Combining Power of Plasma Volumes per 100 ml.	Plasma Chlorides Mg. per 100 ml.
2		62.3	6.4	58.9	600
3	0.2 indirect	80.5	8.3	56 0	595
4		88.9	10.7		
6		125.0	13.0	45.7	585
7		141.4	15.5	35.2	
9		164.0	14.0	31.5	
10 DIED		193.4	17.6		

The patient was treated by intravenous injections of isotonic saline solution, blood transfusions, and spinal anesthesia with procaine hydrochloride. No therapeutic result was attributed to any of these measures.

After the hemolytic reaction the blood grouping and crossmatching procedures were repeated with the centrifuge technique but no incompatibility could be demonstrated. The Rh factor was unknown and the bloods were not incubated during the tests. The pertinent laboratory findings are contained in Table XXI.

TABLE XXI

Laboratory Findings in Case 6

Days After Transfusion	Serum Van den Bergh Mg. per 100 ml	Blood Urea Nitrogen Mg. per 100 ml.	Blood Creatinine Mg. per 100 ml.	Urinary Volume in 24 Hours Ml.	
1	7.7 biphasic	53.9	3.4	40	Transfusion
2		67.9	5 0	619	Spinal Anesthesia
3	3.5 direct	73 5	5.0	390	
4		66.5	5.8	1550	
5				3400	Transfusion
6		62 3	5 0	3635	
7	2.3 direct	56 7	3 2	2800	
8		41.3	3.1	1675	
9		24 5		3425	
10				2150	
13 RECOVERY		9.8	1.2		

Case 44-6538.[5] A woman, aged 30 years, belonging to group B, had never been pregnant. She had received three blood transfusions eleven and ten years before. Some had been accompanied by undiagnosed febrile reactions. A transfusion of group B blood was given during the last admission to the hospital without reaction. Twelve days later 500 ml. of

blood was transfused during which the recipient was observed very closely. No untoward symptoms occurred until 20 minutes after completion of the injection when she experienced a chill and the rectal temperature rose to 102° F. (38.8° C.). She did not appear seriously ill. Fourteen hours later it was discovered that only a small volume of urine had been voided and this contained free hemoglobin and casts. The skin was flushed and she appeared apathetic. The systolic blood pressure was 60 mm. of mercury and the diastolic pressure was immeasurable. Hypodermic injections of ephedrine hydrochloride restored the blood pressure to normal. The daily urinary volume varied between 50 and 250 ml. The renal pelves were washed with warm water without relief. Solutions of dextrose, saline, and sodium r-lactate were given intravenously. The values for blood urea and creatinine rose daily. On the third day after transfusion decapsulation of both kidneys was performed. The capsules stripped with ease and the renal substance did not appear to be edematous. The urinary output was not augmented, vomiting became more frequent, and the patient died in coma on the eleventh day after transfusion. The last day of life the blood urea nitrogen was 87.5 mg. per 100 ml. and the blood creatinine was 14.5 mg. per 100 ml.

A blood specimen taken fourteen hours after transfusion contained free hemoglobin in the serum but no methemoglobin. Both methemoglobin and hemoglobin were found in the urine specimen obtained at the same time. The patient's erythrocytes were Rh negative and those of the donor were Rh positive. Both bloods belonged to group B. The patient's serum contained an agglutinin which clumped the donor's cells after incubation for sixty minutes at 37° C. This could not be demonstrated by the centrifuge technique without incubation.

AIR EMBOLISM

Embolic phenomena are extremely rare in the transfusion of blood by modern equipment. If the blood is filtered before injection into the recipient, there is slight chance of introducing clots into the circulation. If embolism occurs, the clot lodges in the lungs. Air embolism is slightly more likely in transfusion and may occur in the donor or the recipient.

Etiology. It has been estimated that at least 30 ml. of air can be injected into the vein of an adult without causing serious consequences. The chief mechanisms by which a recipient may suffer air embolism during transfusion are: (*a*) neglecting to displace with fluid the air in the tubing of the giving set before the transfusion is started; (*b*) neglecting to clamp off the tubing after the blood has run into the vein; (*c*) air may rush into the vein from the flask after the blood has been injected during transfusion under positive pressure; (*d*) neglecting to cover an erect line or flask filter which becomes partly occluded (p. 531).

When air embolism occurs, the air is carried to the right auricle and thence to the right ventricle. If the volume of air is small, it is pushed through the pulmonary arteries into the arterioles and capillaries of the lungs, where little damage is done. When the volume of air is greater, it is caught in the outlet of the right ventricle and thus blocks the flow of blood to the lungs, causing anoxia. The systemic arterial pressure is consequently reduced and the venous pressure is raised because of the overfilled right heart.

Incidence. This complication is rare in transfusion but it may occur in either recipient or donor.

Clinical Description. During transfusion the recipient may notice a peculiar sensation which one has likened to a feeling of "water and air mixed in a bottle."[37] Sudden, severe cyanosis develops and dyspnea becomes extreme. An observer, as well as the patient, may hear a gurgling or clicking sound in the heart. With smaller amounts of air, several dull thuds may be heard in the precordium. When obstruction to the outflow of the right ventricle occurs, the systemic blood pressure diminishes and the peripheral veins become distended from the increased venous pressure. A "mill-wheel murmur" may be heard in the heart. The symptoms and signs usually subside promptly if the volume of injected air is not great; otherwise death occurs rapidly.

Diagnosis. The condition may be recognized from the clinical description, but it must be differentiated from circulatory overload.

Treatment. The patient should be placed lying on the *left* side. In experiments on dogs Durant *et al.*[38] have recently shown that the position of the animal greatly affects the chances of the heart expelling the air. If the animal is lying on its right side with a sufficient volume of air in the right ventricle, the air rises and obstructs the outlet. If the animal is placed on its left side, the air floats up from the outlet, permitting passage of blood. The air remains in the ventricular chamber where it is churned into foam with the blood and is subsequently expelled into the lungs in small amounts.

Prognosis. This depends upon the amount of air injected. The outlook for rapid recovery is probably good if the patient has received less than 30 ml. of air rapidly.

Prophylaxis. The condition can be prevented by the use of proper equipment and the careful training of personnel in the operation of apparatus.

EXSANGUINATION

From the observation of minor accidents in transfusion the authors believe that the possibility exists of possible exsanguination

blood was transfused during which the recipient was observed very closely. No untoward symptoms occurred until 20 minutes after completion of the injection when she experienced a chill and the rectal temperature rose to 102° F. (38.8° C.). She did not appear seriously ill. Fourteen hours later it was discovered that only a small volume of urine had been voided and this contained free hemoglobin and casts. The skin was flushed and she appeared apathetic. The systolic blood pressure was 60 mm. of mercury and the diastolic pressure was immeasurable. Hypodermic injections of ephedrine hydrochloride restored the blood pressure to normal. The daily urinary volume varied between 50 and 250 ml. The renal pelves were washed with warm water without relief. Solutions of dextrose, saline, and sodium r-lactate were given intravenously. The values for blood urea and creatinine rose daily. On the third day after transfusion decapsulation of both kidneys was performed. The capsules stripped with ease and the renal substance did not appear to be edematous. The urinary output was not augmented, vomiting became more frequent, and the patient died in coma on the eleventh day after transfusion. The last day of life the blood urea nitrogen was 87.5 mg. per 100 ml. and the blood creatinine was 14.5 mg. per 100 ml.

A blood specimen taken fourteen hours after transfusion contained free hemoglobin in the serum but no methemoglobin. Both methemoglobin and hemoglobin were found in the urine specimen obtained at the same time. The patient's erythrocytes were Rh negative and those of the donor were Rh positive. Both bloods belonged to group B. The patient's serum contained an agglutinin which clumped the donor's cells after incubation for sixty minutes at 37° C. This could not be demonstrated by the centrifuge technique without incubation.

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SERUM SICKNESS

A syndrome resembling serum sickness has been rarely observed after blood transfusion.

Etiology. The cause and the mechanism are unknown. In both cases observed by the authors there was no history of injection of animal serum into either donors or recipients. Neither recipients or donors were receiving drugs which are known to produce similar toxic symptoms. There was no history of allergic manifestations in the persons involved.

Clinical Description. The incubation was five days in one case and twelve days after transfusion in the other. The recipients developed fever, urticaria, swollen painful joints, and tender enlarged lymph nodes in the neck, axillae, and groins. The manifestations lasted four or five days and then subsided completely.

Diagnosis. The condition can be recognized from the clinical symptoms and signs. The incubation period is typical. The possibility of the injection of animal serum should be excluded. Toxic symptoms from drugs should be considered.

Treatment. Epinephrine hydrochloride in doses of 0.3 mg. may be given subcutaneously for urticaria. Benadryl or pyribenzamine in 50-mg. doses may be administered by mouth three or four times daily.

Prognosis. Good for complete recovery.

Prophylaxis. This is unknown.

TRANSMISSION OF DISEASE

Theoretically many infections can be transmitted by blood transfusion. The practice of accepting as blood donors only those persons who state that they are in good health and whose appearance confirms it, eliminates the transmission of most infections. There remain a relatively few conditions in which the organisms may exist in the blood stream without causing symptoms at the time when the person presents himself for the donation of blood.

Syphilis. During the period of spirochetemia syphilis may be transmitted by transfusion. The disease has manifestations similar to those encountered in the patient who acquires the infection by contact, with the following differences: (*a*) there is no chancre; (*b*) the incubation period is one to four months after transfusion, with an average of eight to ten weeks; (*c*) the eruption of the secondary stage is said to appear first on the extremities.

The diagnosis is made from the clinical manifestations of the disease and the characteristic reactions of complement fixation

of a recipient during blood transfusion, although such an instance is unknown to us.

Etiology. Occasionally during transfusion the rubber tubing, through which the blood runs from the flask to the patient's vein, becomes separated from its attachment on the flask and falls to the floor. This provides an outlet through which the recipient's blood flows out onto the floor. If the patient is too ill to call an attendant, serious loss of blood could occur from this accident.

Clinical Description. It is conceivable that the loss of blood might be sufficient to cause hemorrhagic shock. The description, diagnosis, and treatment of this condition are discussed in Chapter 3.

Prophylaxis. All connections on the giving set should be securely fastened. The patients receiving transfusions or intravenous infusions should be frequently observed.

GROSSLY CONTAMINATED BLOOD

A few accidents have been observed from the transfusion of blood which has been grossly contaminated.

Etiology. No bacteriologic data are available to determine whether the syndrome is caused by a specific organism.

Incidence. Fortunately this complication is extremely rare and does not occur with the careful handling of blood, although most workers almost never culture flasks of preserved blood in a bank.

Clinical Description. During or soon after transfusion, the recipient becomes violently ill with chills, fever, nausea, and vomiting. There is great prostration, collapse, and severe diarrhea with bloody stools. The patient dies within a few minutes or hours.

Diagnosis. The condition can be suspected from the symptoms and signs. Examination of the residuum of donor's blood will usually reveal evidence of contamination so great that organisms can be seen microscopically on direct inspection of a slide preparation. The blood may have a fecal odor.

Treatment. Specific therapy is unknown. Symptomatic treatment is indicated. If there is evidence of primary shock drugs should be given which cause vasoconstriction.

Prognosis. Probably fatal.

Prophylaxis. Ordinary aseptic technique in handling preserved blood will undoubtedly prevent this complication. It is stated that grossly contaminated blood can sometimes be recognized by the violet color of the cell layer in the flask and by the fecal odor. The present authors have had no experience with this.

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Etiology. The cause and the mechanism are unknown. In both cases observed by the authors there was no history of injection of animal serum into either donors or recipients. Neither recipients or donors were receiving drugs which are known to produce similar toxic symptoms. There was no history of allergic manifestations in the persons involved.

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Prognosis. Good for complete recovery.

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Theoretically many infections can be transmitted by blood transfusion. The practice of accepting as blood donors only those persons who state that they are in good health and whose appearance confirms it, eliminates the transmission of most infections. There remain a relatively few conditions in which the organisms may exist in the blood stream without causing symptoms at the time when the person presents himself for the donation of blood.

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The diagnosis is made from the clinical manifestations of the disease and the characteristic reactions of complement fixation

and flocculation tests such as the Wassermann and Kahn procedures.

The treatment of transfusion syphilis is the same as that given when the disease is acquired by other means.

The prophylaxis is accomplished by (*a*) the rejection of seropositive blood for transfusion, (*b*) the examination of the genitalia and skin of prospective blood donors for chancres and secondary exanthemata, (*c*) the transfusion of blood which has been refrigerated for at least three days. Serologic tests for syphilis will not eliminate all donors who have the disease. In fact, the blood of the donor recently infected with *Treponema pallidum* is most likely to transmit the organism before the serologic tests become positive. In a sense, therefore, Wassermann-negative blood is more dangerous than that which reacts in the test. Physical examinations should be performed on prospective donors to detect primary and secondary manifestations of the disease in the dangerous period before the blood becomes seropositive. The male genitalia should be examined particularly for chancres, but in most clinics the female genitalia are not inspected because of the notorious difficulty in finding the lesions. Probably the best preventive against transmission of syphilis by transfusion is the employment of preserved blood. Turner and Diseker[39] have shown that *Treponema pallidum* does not survive refrigeration for three days.

Malaria. The malaria plasmodia may survive in the body of infected persons for many years without producing symptoms. Furthermore the parasites may be present in such small numbers that the most assiduous search of blood films will not reveal their presence.

The clinical manifestations of malaria usually appear from five to thirty days after transfusion, although cases have been reported with an incubation period as short as one and as long as sixty days. Most of the cases of transfusion malaria have occurred from *Plasmodium vivax*.

The treatment of transfusion malaria is similar to that acquired by the bite of the mosquito. The prognosis for complete cure may be somewhat better because the disease will be recognized early if the physician is alert to the possibility.

In some respects the prophylaxis of transfusion malaria is unsatisfactory. The parasites survive storage in the refrigerator as long as the erythrocytes, so that the preservation of blood is no protection. The physical examination of prospective donors and inspection of films of their blood for parasites do not exclude the possibility of transmission of the disease. Workers in the United States have advocated the rejection of all donors who have lived

in regions where malaria is endemic. Since World War II, when so many soldiers were exposed to malarial infections or had the disease, some authors have accepted as donors those persons who have been free from symptoms for two years without the influence of malarial suppressants. Further experience is needed to determine whether this measure is adequate. The methods of prophylaxis suggested for temperate climates are obviously no solution at all for those who must operate blood transfusion services in the endemic malarial regions of the world.

Infectious Hepatitis (Homologous Serum Type). During World War II it was found that certain lots of supposedly normal human serum which were added in small quantities to yellow fever vaccine produced infectious hepatitis when given to members of the armed forces. Later the same disease was observed to occur from the infusion of dried pooled plasma. The studies of Neefe *et al.*[40] suggest that the disease is caused by a filterable virus which is distinct from that which produces the epidemic type of infectious hepatitis. They gave successive inoculations of infective material from patients with both diseases to groups of human volunteers and demonstrated that an attack of one disease did not protect against the other, although the first attack protected against subsequent inoculations with the same infective agent. Furthermore, the incubation period of epidemic hepatitis is said to be fifteen to thirty-four days whereas that of homologous serum jaundice has an incubation period of fifty-six to 134 days.[41] Both diseases may be transmitted by the serum from infected persons. The infective agent of epidemic hepatitis has been demonstrated in the feces of patients with the disease, but a transmissible virus has not been encountered in the excreta of those with homologous serum jaundice.

Clinically, the onset is usually insidious, with gradually increasing malaise, nausea, vomiting, and slight fever. Frequently there is some diarrhea with excessive flatus. Pain is elicited when the lower ribs are percussed in the anterior axillary line on the right side. The liver may be tender and palpable; splenomegaly frequently is present. Icterus is usually seen although it may be absent. The diagnosis is made by the demonstration of an infectious hepatitis[42] in a patient who has received human whole blood or blood derivatives within the known incubation period. The mortality has been variously estimated at between 0.2 and 10 per cent. The treatment of this disease is not within the scope of the discussion.

In the past the only prophylactic measure has been to reject those persons who have had jaundice within six months of the time they

and flocculation tests such as the Wassermann and Kahn procedures.

The treatment of transfusion syphilis is the same as that given when the disease is acquired by other means.

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process of crystallization. Solutions of this material will give pyrogenic reactions when employed in blood transfusion. In purchasing the chemical for intravenous use the manufacturer should be requested to state that it was prepared in such a way as to eliminate the possibility of pyrogen formation.

Binding of Calcium in Recipient's Plasma. With the transfusion of large amounts of citrated whole blood or plasma signs of hemorrhage or tetany have rarely been observed. Several data explain the innocuousness. Neuhof and Hirshfeld[43] injected adults intravenously with solutions containing as much as 8 gm. of sodium citrate in ten minutes without producing symptoms. This is probably a larger dose than when the same amount of citrate is first added to plasma or whole blood and then transfused, because some of the citrate radical binds the calcium of the donor's blood and is therefore not available to act upon the plasma of the recipient. The rate of injection of sodium citrate must be very rapid to produce any untoward effects. Salant and Wise[44] have shown that 90 per cent of the citrate radical is removed from the blood stream in ten minutes and it is rapidly oxidized and excreted from the body.

Isolated instances have been observed in which tetany occurred when newborn babies were transfused with citrated whole blood or plasma. In such a situation it is wise either to avoid the use of citrate or to inject calcium gluconate intravenously at intervals during transfusion, such as in replacement transfusion in the treatment of erythroblastosis fetalis. The injections of calcium should be made separately and *not* added to the citrated blood. The occurrence of tetany in adults from even large volumes of citrated whole blood or plasma in transfusion is probably extremely rare (see p. 434).

During the treatment of a patient with hemorrhagic shock by transfusions of citrated blood or plasma, the observation is occasionally made that the patient's blood does not clot properly. This has been ascribed to the elaboration of heparin in the liver which occurs in hemorrhagic shock.

ISOSENSITIZATION AS A COMPLICATION

The incidence of isosensitization as a complication of blood transfusion will not appear in any series of immediate transfusion reactions but is usually a remote result whose manifestations may occur years later, either as hemolytic reactions to subsequent transfusions or in the occurrence of hemolytic disease of the newborn. The subject is discussed in detail in Chapter 8.

present themselves for the donation of blood. This precaution has not proved efficacious, as attested by the fact that many cases of the disease have been reported in recipients of plasma or whole blood from donors who were screened in this manner. The incidence of the disease in the general population is unknown. It is recognized that some persons have the disease without exhibiting easily identified signs such as jaundice. There is no known method of excluding donors who are incubating the disease when they give blood, although the blood may be infectious at that stage. Furthermore there is some evidence that many persons may be carriers of the virus without actually developing the disease in recognizable form.

It is possible that some cases of homologous serum jaundice might be prevented by excluding all donors who exhibit costal tenderness over the liver, splenomegaly, hepatomegaly, or clinical icterus. Donors who have received blood or blood derivatives within six months could be rejected to exclude the rare person who might be infected in this manner. Discarding blood in the bank when the plasma exhibits an abnormally high concentration of bilirubin might be a practical procedure. From what is known of this disease it is too much to hope that these measures would prevent all cases of homologous serum jaundice. The incidence of the disease is much greater when pooled plasma is used than when whole blood is transfused, as one infected bleeding will contaminate an entire pool of plasma (p. 356).

Other Diseases. Rare complications of blood transfusion have been the transmission of virus infections such as smallpox, measles, and influenza. Any organism which causes a bacteremia can be transmitted. One reason for rejecting as donors persons who appear to have the symptoms of a common cold is that they may be having the prodrome of any of a number of contagious diseases.

REACTIONS FROM SODIUM CITRATE

Ever since the introduction of sodium citrate as an anticoagulant a controversy has existed with regard to the toxicity of the compound when injected in transfusion. Most reactions ascribed to the citrate radical were observed before the discovery and elimination of pyrogens from fluids and apparatus. With newer methods of cleaning and preparing equipment for transfusion the prejudice against citrated blood has largely disappeared. Complications from sodium citrate are seldom observed but, when encountered, they may be classified in one of the following categories:

Pyrogens in Crystalline Sodium Citrate. Occasionally a lot of sodium citrate becomes contaminated with pyrogens during the

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(c) DeGowin, E. L., Warner, E. D., and Randall, W. L.: Anatomic changes in man compared with those in dogs with experimental hemoglobinuria. *Ibid* 61:609, 1938.

FACTORS ERRONEOUSLY ASCRIBED AS RELATED TO COMPLICATIONS

Speed Shock. A syndrome, popularized under the name of speed shock, was described by Hyman and Hirshfeld.[45] It was thought that the rapid injection intravenously of many nontoxic substances could produce severe symptoms or sudden death. This theory has not been substantiated by subsequent investigators and the reactions observed after the rapid injection of whole blood are probably caused by circulatory overload, pyrogens, or hemolysis.

Retinal Hemorrhages. In the older literature may be found many articles in which retinal hemorrhage is described as a complication of blood transfusion. Careful scrutiny of the reports reveals that most of the recipients in whom retinal hemorrhages were observed after transfusion had blood dyscrasias in which the complication is likely to occur spontaneously without transfusion. The consensus favors the view that properly conducted blood transfusion probably does not cause this complication.

Transfusion of Cold Blood. Formerly it was believed that the intravenous injection of cold blood or other fluids produced reactions. Most observations to support this contention were made before pyrogens were excluded. DeGowin, Hardin, and Swanson[46] transfused blood mixtures with temperatures at least 10°C. below those of the room, with velocities up to 40 ml. per minute. No untoward reactions were observed and the body temperature of the recipients was not significantly affected.

Hematuria. The occurrence of erythrocytes in the urine of the recipient cannot be regarded as a complication of transfusion. In examining posttransfusion urine specimens it is essential to differentiate between hematuria and hemoglobinuria as the significance of each is entirely different.

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The minimum concentration of trisodium (dihydric) citrate which is generally considered acceptable to prevent coagulation in a blood mixture is about 0.25 per cent. The permissible maximum concentration is not accurately known. It has been demonstrated that as much as 8 gm. of sodium citrate in solution can be administered intravenously to an adult in ten minutes without producing tetany (p. 297).

Crystals of sodium citrate for use in blood transfusion should be specified by the manufacturer as being pyrogen-free because pyrogens can be incorporated into the compound during crystallization when certain manufacturing processes are used..

Disodium Citrate. Loutit and Mollison[3] introduced the use of disodium monohydric citrate as an anticoagulant in blood preservative mixtures. This is an acid salt, furnishing a maximum amount of citrate ion, which may be autoclaved in the same solution with dextrose without causing caramelization of the latter. If the alkaline trisodium citrate is heated with dextrose solutions, caramelization occurs unless the pH is adjusted to 7.0 or less by the addition of citric acid or phosphate buffers. The disodium citrate is not readily available in the United States in a form free from pyrogens.

Heparin. Heparin is available as an anticoagulant for use intravenously. It is more expensive than citrate compounds and possesses no distinct advantage over them. Furthermore its anticoagulant activity diminishes during the storage of the blood so that the action cannot be relied upon. The deterioration of erythrocytes in heparinized blood during storage is more rapid than in citrated blood[14] and therefore the substance is little used in transfusions.

BLOOD STORED IN SODIUM CITRATE

The changes in citrated blood stored at 2° to 8° C. will first be described because this blood mixture is the simplest used in the bank and, in general, deterioration progresses at the most rapid rate. All methods of preservation are designed as improvements over the storage of whole blood in a solution of sodium citrate.

The Gross Appearance

When 500 ml. of blood is collected in a flask containing 50 to 75 ml. of 3.2 per cent solution of trisodium dihydric citrate, a dark red opaque mixture is formed. The flask is placed upright on the shelf of a refrigerator adjusted to maintain the temperature between

CHAPTER 13

Storage and Preservation of Whole Blood

By Elmer L. DeGowin

ANTICOAGULANTS
BLOOD STORED IN CITRATE SOLUTION
PRESERVATION OF BLOOD
BLOOD PRESERVATIVE MIXTURES

The idea of storing blood for transfusion originated with Rous and Turner[1] and much of the early work on the preservation of erythrocytes was published by them in 1916. The validity of their observations has been amply confirmed by others. Many subsequent authors on the subject of blood preservation would have avoided certain pitfalls had they carefully perused these early publications. The first transfusions of preserved blood were given to human beings by Robertson[2] who applied the method of Rous and Turner in the operation of the first blood bank. This was established in casualty clearing stations of the British army in 1917. From this beginning, the use of stored blood has gradually become the salient feature of transfusion therapy.

ANTICOAGULANTS

One of the necessary prerequisites to the storage of blood is the addition of a substance which prevents coagulation. The anticoagulant must not cause deterioration of the cells and it must be nontoxic to the recipients of transfusion. Defibrinated blood deteriorates more rapidly than that to which anticoagulants are added.

Trisodium Citrate. The anticoagulant most commonly employed is trisodium citrate. It is usually available in two forms, and in making blood preservative solutions care must be taken to incorporate the correct salt given in the formula because the two compounds are not chemically equivalent by weight. The compound $2Na_3C_6H_5O_7 + 11H_2O$ is isotonic with plasma in a concentration of 3.8 per cent, whereas the dihydric salt $Na_3C_6H_5O_7 + 2H_2O$ produces an equivalent isotonicity in a 3.2 per cent concentration.

The disintegration of erythrocytes releases free hemoglobin which colors the plasma red. In the stationary flask of blood the free hemoglobin diffuses slowly into the plasma layer so that considerable hemolysis may occur before the undisturbed supernatant plasma is colored. This fact was first observed by Rous and Turner.[1] After a few days of storage the lower portion of the plasma becomes pink. This layer of color gradually increases in height in the plasma column, the portion at the erythrocyte interface becoming deeper red. Finally the entire plasma layer becomes red. It should be evident from the description that an accurate estimate of the amount of free hemoglobin in the blood mixture can only be made by thoroughly mixing the cells and plasma and analyzing a sample of plasma separated by centrifugation. As time passes the plasma turns a deeper red and the erythrocyte layer becomes darker and its plasma interface indistinct.

The Erythrocytes

Since the primary purpose of transfusion is to supplement the erythrocytes of the recipient with those of another, the red cells have received the most attention in studies on storage and preservation.

Cytologic Changes

In any microscopic preparation of red cells in stored blood the observer sees erythrocytes which are apparently normal in shape and volume, others which have increased in size and assumed the form of spheroids, while still others are crenated. An enumeration of the proportion of various forms is probably unprofitable because no conclusions can be drawn. Furthermore, during storage there seems to be a tendency for increased cohesion between red cells which is evident in all preservative mixtures, but especially those in which dilution of plasma is considerable.

Spontaneous Hemolysis

The determination of the concentration of free hemoglobin in the plasma is probably the most accurate method of gauging the number of red cells destroyed during storage. It has been argued that erythrocytes may lose their hemoglobin and still remain intact morphologically, but most workers have assumed that free hemoglobin in the plasma is derived from ruptured erythrocytes.

If delicate tests are employed, it can be shown that a small amount of free hemoglobin is always present in the plasma after the collection of 500 ml. of blood by the procedures ordinarily

2° and 8° C. The red cells begin to settle out in the fluid so that between twenty-four and forty-eight hours maximum sedimentation has nearly been attained. Some blood specimens settle much more rapidly than others. At this stage the flask contains a column consisting of a bottom layer colored dark red and completely opaque. This is composed of erythrocytes which have gravitated to the bottom of the container. The depth of this layer depends upon the hematocrit of the donor but, in general, is about two fifths of the height of the column. The upper three fifths is made up of a layer of citrated plasma which is light yellow to straw-colored. It may be quite transparent, but the presence of minute globules of lipids causes various grades of opalescence, from a slight opacity to a frankly milky appearance. With properly collected blood there is no visible red color in the plasma layer.

If the plasma is clear, a thin layer of grayish material rests on the upper surface of the erythrocyte column. This is usually a fraction of a millimeter in thickness and is composed of leukocytes and platelets. It is referred to as the *buffy layer* or the *leukocyte cream.*

If fibrin forms in the blood mixture, it takes one of three shapes. Large clots are usually immersed in the erythrocyte layer from which a few fibrin strands lead up into the plasma and are attached to the walls of the flask, or are fixed to a pellicle floating on the surface of the plasma. These strands usually contain sufficient entrapped erythrocytes to make them conspicuous with a faint red color. Fibrin may form as filmy strands, without entrapped red cells, which crisscross the plasma layer in varigated patterns which have the appearance of sheets or veils. These structures are usually formed after the cells have sedimented and are more difficult to see because they contain no erythrocytes. Frequently the only visible fibrin in the flask is in the form of a bit of foam which floats on top of the plasma layer and contains few red cells. This is usually the result of a small clot forming at the conclusion of the blood collection or developing on the walls of the flask where blood has not been reached by the anticoagulant.

As storage proceeds the opacity of the citrated plasma gradually increases. This is caused in some cases by the coalescence of lipid globules and the precipitation of plasma globulin. At times the particles become granular so that when the plasma is disturbed, whirls are caused which strikingly resemble those seen when bacteria grow in liquid media. If the lipid globules are large, they float to the top of the plasma layer.

With aging the buffy coat or layer becomes more compact and the ends tend to curl so that it may resemble the growth of some molds in liquid media.

MECHANICAL FRAGILITY

Rous and Turner[1] first pointed out that there was no relationship between the osmotic fragility of stored erythrocytes and their ability to withstand shaking without rupture. Quantitative measurements of the resistance to trauma are difficult to make accurately but rough estimations have been published.[8] Apparently the mechanical fragility of erythrocytes in citrated blood increases considerably during storage. More accurate and detailed information on this property of red cells seems desirable. The normal erythrocyte is subjected to considerable buffeting and squeezing every hour that it stays in the circulation, as it collides with other cells, is whisked into various channels, and forced through the lumens of small vessels. Mechanical fragility may well be an essential quality of the well-preserved red cell.

GLYCOLYSIS

In defibrinated blood kept at 37° C. Guest[11] reported that the concentration of dextrose diminishes at the rate of 13 to 16 mg. per 100 ml. per hour, until a level of approximately 20 mg. remains. When this minimum is attained, the content of inorganic phosphorus begins to rise sharply and progressively at the expense of the cellular acid-soluble phosphorus. When citrated blood is stored at 2° to 8° C. the same process of glycolysis occurs but at a slower rate. Various studies[8,9,12,13,14,15] show disappearance rates of from 5 to 13 mg. of glucose per 100 ml. per twenty-four hours, for the first five days of storage. Thereafter the rate of glycolysis is slower but the dextrose is quickly exhausted. The increase in the inorganic phosphorus of the erythrocytes becomes accelerated as the sugar disappears. There is a steady rise in the lactic acid of the plasma as glycolysis proceeds, so that concentrations of 100 to 160 mg. per 100 ml. are attained. The pH of the blood, however, is affected but little. There is a slow increase in the reduced glutathione.[12] Many observers have noted that there is an acceleration of the rate of spontaneous hemolysis at the time when the dextrose of the menstruum is exhausted.

POTASSIUM OF THE ERYTHROCYTES

Human red cells contain from seventeen to twenty times as much potassium as is found in normal plasma. Dulière[16] first noted that during storage the cellular potassium diffused into the plasma. This phenomenon has been the subject of extensive investigation.[5,10,17,18] The main facts are agreed upon. There is rapid diffusion of potassium during the first five days, thereafter the rate is somewhat slower, and the maximum concentration is attained in the plasma

used in transfusion. In thirty-nine such bleedings DeGowin and Hardin[4] obtained a range from 1.0 to 9.7 mg. of hemoglobin per 100 ml. of plasma. The concentration of 10 mg. of hemoglobin per 100 ml. of plasma is barely perceptible as a faint pink color. The hemoglobin concentration in the plasma is no greater when the blood is collected in evacuated flasks.

In studies made on citrated blood stored at 2° to 8° C.[5,6] the concentration of plasma hemoglobin was determined after the cells and plasma were thoroughly mixed and then separated by centrifugation. For six blood mixtures the ranges were: no days, 0 to 1.0 mg. per 100 ml. of plasma; five days, 8 to 27 mg.; ten days, 57 to 214 mg.; fifteen days, 165 to 524 mg.; and in two bloods at twenty days, 460 and 661 mg.; twenty-five days, 1060 and 1253 mg.; and at thirty days, 1503 and 2223 mg. In another study of two bloods[7] the erythrocytes lost during storage were estimated from the plasma hemoglobin concentration as follows: five days, 0.09 and 0.17 per cent; ten days, 0.73 per cent; fifteen days, 1.51 per cent; twenty days, 3.58 and 3.24 per cent; twenty-five days, 7.20 per cent; and thirty days, 13.80 and 9.08 per cent.

Osmotic Fragility

The changes in osmotic fragility of the erythrocytes of citrated blood during storage have been studied by a number of investigators and the results agree. A representative protocol[8] of a stored citrated blood is given in which are expressed the maximum concentration of sodium chloride solution in which hemolysis occurred and also the highest concentration in which complete hemolysis was present: no days, 0.40 to 0.32 per cent; five days, 0.64 to 0.32 per cent; fifteen days, 0.64 to 0.32 per cent; twenty days, 0.88 to 0.40 per cent; twenty-five days, 0.88 to 0.40 per cent; thirty days, 0.92 to 0.44 per cent. The increase in fragility indicates an augmentation of cell volume which was confirmed by the hematocrit readings. The cell volume showed the following increases over the initial: 13.8 per cent on the fifteenth day; 21.4 per cent on the twentieth day; 31.5 per cent on the twenty-fifth day; and 47.0 per cent on the thirtieth day.

The significance of the changes in osmotic fragility in citrated blood during storage has not been completely explained. The increase in cell volumes parallels the augmentation of intracellular inorganic phosphorus,[9] the loss of potassium from the erythrocytes,[10] and the increase in spontaneous hemolysis. But these phenomena occur at approximately the same rates in blood stored in sucrose solutions in which the osmotic fragility is actually less than normal.[8]

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between the fifteenth and twentieth day. There is no relation between the rate of potassium loss and the rate of spontaneous hemolysis during storage. The loss of potassium from the erythrocytes does not seem to have any practical bearing on blood preservation.

Agglutinogens

It is the general impression that the sensitivity of isohemagglutinogens survives storage well, although no quantitative measurements are available to support this conclusion.

Oxygen Capacity

The oxygen-carrying capacity of the red cells diminishes little, if at all, during the time of storage.[13,15,19,20]

The Leukocytes

Rate of Deterioration

The composite results of several workers[13,15,21] indicate that the total number of leukocytes has decreased about two thirds by the third day of storage, although one group[22] have summarized the rate of loss as follows: first day, 11 per cent; second day, 17 per cent; fourth to fifth day, 22 per cent; sixth to tenth day, 24 per cent. Many of the enumerated cells have become so degenerated as to be nonfunctional.

Cytologic Changes

Most workers agree that the polymorphonuclear leukocytes are the first granulocytes to degenerate. These are followed by the eosinophilic leukocytes and the monocytes. The lymphocytes are the most hardy, persisting for as long as fifty days.[23]

Crosbie and Scarborough[22] have described the morphologic changes in detail. The leukocytes clump after the first twenty-four hours of storage. On the second and third days the nuclei become swollen and frequently elongated, with loss of interlobar bands of chromatin. Granulation and vacuolization of the cytolasm occur. The nuclei finally disappear, leaving outlines typical of basket cells. The motility of the leukocytes may be demonstrated in suitable preparations up to sixteen days, although it is not vigorous after three or four days.

The lymphocytes show changes in eight or ten days. The nuclei become pyknotic and the volume of cytoplasm is diminished. Finally, after fifty to one hundred days, these cells may be seen as dense black dots.

PHAGOCYTIC ACTIVITY

Kolmer[23] has reported observations on the ability of leukocytes in stored blood to ingest bacteria. The phagocytic activity for staphylococci, streptococci, and *B. coli* is practically unimpaired for about three days, but there is rapid diminution after that time.

The Platelets

The thrombocytes survive storage poorly. Three groups of observers[13,14,21] agree that there is a precipitous fall in the count during the first three days, indicating a loss of about two thirds of the platelets. Thereafter the other third slowly diminishes so that counts of 50,000 per cu. mm. are the average. Since there is no satisfactory method of titration of thromboplastin, the physiologic activity of the platelets has not been investigated during storage.

The Plasma

Studies on prepared plasma will not be considered in this discussion which will be limited to plasma stored in contact with erythrocytes. As has been described, during storage the content of potassium and inorganic phosphorus is increased by diffusion from the red cells. The disintegrating leukocytes and platelets may release enzymes and thromboplastin to the plasma. The content of free hemoglobin gradually rises.

PLASMA PROTEINS

Scudder[24] studied the electrophoretic patterns of the plasma proteins in fresh and preserved plasma refrigerated up to fifty-three days and concluded that only minor changes occurred in the albumin, the alpha, beta, and gamma globulins, and the fibrinogen.

PROTHROMBIN

Some discrepancies are noted in the results of various investigators as to the rate at which the prothrombin diminishes in stored blood. In general, the titer obtained with Quick's prothrombin time procedure has been somewhat lower than that with the quantitative method of Warner, Brinkhous, and Smith. Two groups of workers[25,26] who employed the latter method reported similar results. The prothrombin titer gradually diminished during storage so that it attained about 50 per cent of normal in twenty-one days. The rate of disintegration accelerated somewhat after that time. Warner, DeGowin, and Seegers[26] noted no relationship between

between the fifteenth and twentieth day. There is no relation between the rate of potassium loss and the rate of spontaneous hemolysis during storage. The loss of potassium from the erythrocytes does not seem to have any practical bearing on blood preservation.

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CRITERIA OF PRESERVATION

Most investigators have hoped for the development of *in vitro* tests to establish reliable criteria of erythrocyte preservation, because of the relative ease and speed with which they can be performed. The tests most studied for this purpose are: the rate of spontaneous hemolysis during storage, the osmotic fragility of the erythrocytes, and the mechanical fragility of the red cells. Mollison and Young[17] have emphasized the lack of correlation between *in vitro* tests on preserved erythrocytes and the rate of survival of these cells in the recipient after transfusion. They concluded that transfusion survival was the only sound criterion of the efficacy of preservation.

Spontaneous Hemolysis as a Criterion. It is obvious that a method of preservation which permits rapid spontaneous hemolysis during storage is undesirable. The transfusion of blood with much free hemoglobin in the plasma is attended by danger to the recipient. A low rate of hemolysis is therefore a prerequisite to good preservation. But methods of preservation which effectually inhibit spontaneous hemolysis during storage do not necessarily insure good transfusion survival of red cells.

Osmotic Fragility of Erythrocytes as a Criterion. The osmotic fragility of the red cells is admittedly not a good criterion of preservation because of the many exceptions which must be explained. The tests have a certain value in that cells whose osmotic properties are so altered during storage as to cause them to hemolyze in isotonic saline solution are unsuited for transfusion[8] (p. 321). The results obtained in the study of osmotic fragility of the erythrocytes stored in dextrose solutions are not significant for some purposes unless the cells have been washed free from sugar before testing in saline solutions (p. 321). Cells stored in citrate-sucrose solutions have misleadingly low osmotic fragility, which bears little relation to their other characteristics (p. 321).

Transfusion Survival of Erythrocytes as a Criterion. There can be no doubt that measurement of the survival of transfused red cells in the circulation of the recipient is the best criterion of blood preservation. It is, in effect, the biologic test. Unfortunately, the procedures employing either differential agglutination or the determination of radioactive iron (pp. 200 and 208) are so difficult and time-consuming that sufficient data have not been accumulated to evaluate many of the preservative mixtures.

Of the two general methods of measuring transfusion survival, the differential agglutination technique has been more used. In skilled hands the inagglutinable cell counts can be made with an

the rate of diminution of prothrombin and the deterioration of the erythrocytes during storage. It seems probable that transfusions of stored blood will supplement a prothrombin deficiency in the recipient almost as well as fresh blood.

COMPLEMENT

Sodium citrate in stored blood was found to be only slightly uncomplementary by Kolmer[23] and the complement was well preserved during storage from fourteen to twenty-one days.

ISOHEMAGGLUTININS

The titer of anti-A and anti-B agglutinins seems to be maintained in the plasma of stored blood in the original potency for about ten days, according to two groups of observers.[13,21] There is slight impairment in fifteen days, but at twenty days the potency is only half the original.

DIPHTHERIA ANTITOXIN

Rosenthal *et al.*[16] investigated the natural diphtheria antitoxin found in some specimens of stored blood. During the first four months of storage no diminution in potency was noted. After the fifth month there was about 15 per cent loss. They concluded that transfusions of stored blood could be employed in transmitting natural antibodies in the treatment of disease.

PRESERVATION OF BLOOD

Many have employed the terms *stored blood* and *preserved blood* as synonyms. Muether and Andrews[18] were among the first to insist that proper recognition of the difference between the two was important to a logical understanding of the subject. The distinction is indeed helpful and is employed in this discussion. Citrated blood which is kept for some days at from 2° to 8° C. should be designated as *stored blood* because nothing has been added to inhibit deterioration of the erythrocytes. When the rate of disintegration is checked by the addition of other substances to the blood-citrate mixture, the resulting product should be known as *preserved blood.*

Purpose of Preservation. The chief purpose of blood transfusion has been to supply erythrocytes to the patient and therefore the goal of blood preservation is to devise methods by which deterioration of the red cells is slowed or prevented during storage.

Criteria of Preservation

Most investigators have hoped for the development of *in vitro* tests to establish reliable criteria of erythrocyte preservation because of the relative ease and speed with which they can be performed. The tests most studied for this purpose are: the rate of spontaneous hemolysis during storage, the osmotic fragility of the erythrocytes, and the mechanical fragility of the red cells. Mollison and Young[17] have emphasized the lack of correlation between *in vitro* tests on preserved erythrocytes and the rate of survival of these cells in the recipient after transfusion. They concluded that transfusion survival was the only sound criterion of the efficacy of preservation.

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Osmotic Fragility of Erythrocytes as a Criterion. The osmotic fragility of the red cells is admittedly not a good criterion of preservation because of the many exceptions which must be explained. The tests have a certain value in that cells whose osmotic properties are so altered during storage as to cause them to hemolyze in isotonic saline solution are unsuited for transfusion[1] (p. 321). The results obtained in the study of osmotic fragility of the erythrocytes stored in dextrose solutions are not significant for some purposes unless the cells have been washed free from sugar before testing in saline solutions (p. 321). Cells stored in citrate-sucrose solutions have misleadingly low osmotic fragility, which bears little relation to their other characteristics (p. 321).

Transfusion Survival of Erythrocytes as a Criterion. There can be no doubt that measurement of the survival of transfused red cells in the circulation of the recipient is the best criterion of blood preservation. It is, in effect, the biologic test. Unfortunately, the procedures employing either differential agglutination or the determination of [illegible] and time-consumi[illegible] to evaluate many [illegible] preservative mixtures.

Of the two general methods of measuring transfusion survival, the differential agglutination technique has been more used. In skilled hands the inagglutinable cell counts can be made with an

accuracy of ± 5 per cent. The data obtained by Mollison and Young,[27,37] Loutit and Mollison,[3] Loutit, Mollison, and Young,[31] Loutit,[33] and Denstedt *et al.*[28] on blood in various preservative mixtures have been sufficiently consistent to be statistically significant. The last-mentioned group have noted individual differences in recipients in the rates at which they destroy transfused cells. This implies that many data must be obtained to embrace the possible individual variations. Osborne and Denstedt[45] published studies on the details of the procedure using differential agglutination which should improve the accuracy of the method. Differential agglutination has the advantage of permitting repeated observations of the transfused red cells throughout their entire stay in the circulation.

Measurement of transfusion survival of erythrocytes by the radioactive iron technique (p. 208) is the newer method and its use has been quite restricted because of the highly specialized physical procedures involved. Its users have claimed superiorities for the methods which have not been entirely proved. The accuracy is about ± 5 per cent, but the volume of blood transfused has frequently been between 100 and 300 ml., as contrasted with transfusions of 500 to 1040 ml. of whole blood, or 300 to 530 ml. of concentrated red cell suspensions from pooled samples, in the agglutination technique. The cells containing radioiron can be followed in the circulation for only one or two days before a secondary increase in radioactivity of the blood begins, which is ascribed to resynthesis of the tagged hemoglobin into new cells. The measurement of transfused red cells by their radioactivity is usually made about twenty-four hours after injection of the blood. This has been shown to be the *average* time at which most of the cells, which have deteriorated during storage, have left the circulation. Reference to detailed studies will show, however, that this time period sometimes falls on the steep slope of the curve so that slight individual variation in disposing of damaged cells might make a great difference in the apparent value obtained.

From a relatively small number of data Gibson *et al.*[48] constructed mathematical curves in an attempt to evaluate the survival of red cells in various preservatives. An arbitrary value of 70 per cent survival of red cells in twenty-four hours was taken as the minimum measure of acceptable preservation. The day of storage at which the curve, constructed frequently on only four points, intersected the line for 70 per cent survival was used to determine the outdating period of blood in that preservative mixture. This ignores utterly the fact that there is individual variability in blood.

In a cooperative study three groups of investigators attempted the evaluation of some blood preservative mixtures by transfusion. The

radioactivity measurements were all made in the same laboratory. In several instances it is possible to compare the data from the different groups[46,47,48] on the same preservative mixtures. These results are collected in Table XXII. In general, good agreement

TABLE XXII

Comparison of Results of Three Groups of Investigators Using Radioactive Iron Technique on Transfusion Survival of Erythrocytes After Storage in Various Preservatives

Preservative	Investigators	DAYS OF STORAGE																			
		0	1	2	4	5	7	8	10	12	13	14	15	19	20	21	23	24	28	30	31
Citrated Blood	Gibson *et al.*	100					50														
	" " "	52						51													
	" " "		100			90		51													
	Ross *et al.*						50					3				0					
	Range																				
	from	*100*					*50*	*51*													
	to	*52*					*50*	*51*													
A C D Solution	Gibson *et al.*			100					90					90							
	" " "											94							58		
	" " "								99				94			81					
	Strumia *et al.*	90							90						39					11	
	(one donor)	94							91							42					15
	Ross *et al.*						95					92				75				54	
	Range																				
	from	*90*							*90*			*94*				*81*				*64*	
	to	*91*							*97*			*92*				*39*				*11*	
Alsever's Solution	Gibson *et al.*				100					84						70					
	" " "								94				88			62					
	Strumia *et al.*										70										
	(one donor)										64										
	Ross *et al.*						67					60				34			13		
	Range																				
	from										*84*					*70*					
	to										*60*					*31*					
McGill II Solution	Gibson *et al.*	98							96			78				48			22		
	Ross *et al.*						92					90				73			60		
	Range																				
	from											*90*				*73*			*60*		
	to											*78*				*48*			*22*		

(Values in per cent survival of red cells in 24 hours)

was obtained with Alsever's mixture until the thirteenth day of storage and with the ACD and McGill II solutions until the twenty-first day. After these periods the range becomes excessive so that a large number of data would be required from which conclusions could be drawn. Furthermore there are not sufficient observations

on any of the solutions during the critical period between the fourteenth and twenty-first day of storage.

It seems evident that many more observations are necessary with the radioactive technique before sound conclusions can be made as to the relative merits of various blood preservative mixtures. It should also be pointed out that no reports have yet been published of results obtained by combining the agglutination method and the radioactive technique on the same donor's blood injected into the same recipient. The relative merits of the two methods will be in doubt until this is done.

Variability in Blood of Different Individuals

There seems to be considerable variability in the rate of disintegration of the erythrocytes from different persons when blood is collected and stored under similar conditions. In the operation of a blood bank it is observed that some flasks of blood deteriorate more rapidly than others under apparently identical environments. The same variability appears in the more precise studies on blood preservation. Several blood specimens from different individuals have different rates of spontaneous hemolysis when stored side by side in the refrigerator. Failure to appreciate this fact has led to many erroneous conclusions. The individual differences in a small series of blood specimens are sometimes ascribed to the purposely varied conditions of the experiment. In evaluating experimental results it is necessary to have a statistically significant series of observations or, in lieu of this, comparisons should be made under the different conditions of the experiment with aliquots of blood from the same person.

Effects of Various Procedures on Erythrocyte Preservation

The conclusions in this section are often only tentative because of the difficulty in comparing results from different laboratories when the experiments were carried out under slightly different conditions. Then, too, the data in some studies are too few to preclude the possibility that differences may not be due to individual variation in blood.

Varying the Temperature of Storage

All observers agree that spontaneous hemolysis proceeds at a slower rate in the refrigerator than at room temperature. A few quantitative measurements were made of the difference between 5° and 20° C. by DeGowin, Harris, and Plass.[6] The subject was studied in detail by Thistle, Gibbons, Cook, and Stewart[29] who

investigated the rates of spontaneous hemolysis in blood stored at temperatures ranging from $-5.0°$ to $+12.5°$ C. They concluded that minimum hemolysis occurs between 2.5° and 6.0° C. The differences in that range are small. Storage at 0° C. or lower is, of course, undesirable because of the danger of freezing.

CHILLING THE PRESERVATIVE SOLUTIONS BEFORE BLOOD COLLECTION

When blood is withdrawn from the donor for storage, it must undergo the temperature change from 37° to approximately 5° C. A volume of 500 ml. of blood cools rather slowly and unevenly if the flask is merely placed in the refrigerator. The slow rate of cooling and the uneven distribution of heat in the contents of the flask during the transition probably has little effect on citrated blood. If dextrose is present in the blood mixture, however, the sugar may diffuse into the red cells at a rate which produces osmotic hemolysis. It is mandatory that the flask and preservative solution be chilled to the temperature of the refrigerator before adding the blood when one of the more dilute dextrose-citrate mixtures is employed.[6,8] This results in a more rapid and uniform cooling of the erythrocytes (p. 322). There is suggestive evidence that the red cells persist in the circulation longer when they are collected in any solution which is prechilled.

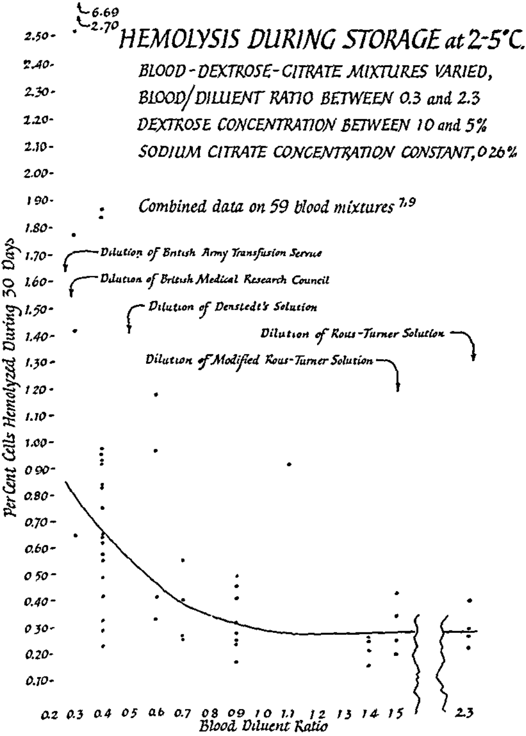
VARYING THE GASEOUS TENSION OF THE BLOOD MIXTURE

Blood was first preserved in flasks stoppered with cotton wool. This permits free access of air to the blood mixture. Later blood flasks were partially filled and closed with stoppers impermeable to gases. This permits a limited gaseous exchange between the blood mixture and the entrapped air. Since hemoglobin combines with more oxygen at lower temperatures, the blood mixture in the refrigerator dissolves oxygen which is released in gas bubbles as the blood runs through the tubing and is warmed up during a transfusion.

Gnoinski[10] first reported that blood was well preserved in ampules which were completely filled so that all air was excluded. DeGowin *et al.*[5,6] compared the rates of spontaneous hemolysis in blood stored in the air and blood mixtures in completely filled, sealed flasks. There was significantly less hemolysis in the blood stored under anaerobic conditions. These findings have been confirmed by others.[29] The tension of oxygen and carbon dioxide in blood stored with the exclusion of air approximates that in the venous circulation and is therefore more nearly physiologic.

The inhibition of hemolysis in stored blood by the exclusion of air is probably not important in itself but the complete filling of

the blood flasks minimizes shaking during transportation and leaves no waste space in the container.



The storage of blood under an atmosphere of carbon dioxide has been advocated by Scudder and Smith[31] who showed that such a procedure slowed the electrolyte diffusion through the cell

membranes. This cumbersome method does not justify the possible advantages inasmuch as better survival of the red cells after transfusion has not been demonstrated. Furthermore no comparison has been made with blood stored with the simple exclusion of air by filling the flask and closing with an impermeable stopper.

Dilution of Blood with Nonelectrolyte Solutions

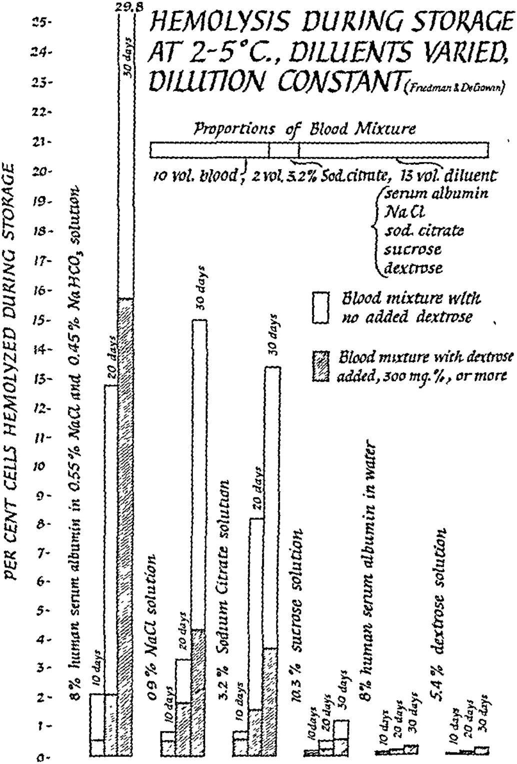
Inhibition of spontaneous hemolysis during storage by dilution of blood with nonelectrolyte solutions was first reported by Rous and Turner.[1] Succeeding writers have considered the factor of dilution but few quantitative data have been presented. In the accompanying graph the amounts of hemolysis in thirty days at 2° C. are plotted for fifty-nine blood specimens. The concentration of sodium citrate was 0.26 per cent in all mixtures. The dextrose concentration was in excess of the minimum requirements for glycolysis (the range was from 1.0 per cent to 5.7 per cent). The blood/diluent ratio varied from 0.3 to 2.3. Analysis of the scatter curve seems to show a slight but definite increase in hemolysis when the blood/diluent ratio was less than 0.9. When plasma was used as a diluent, Parpart *et al.*[42] concluded that a blood/diluent ratio of 0.4 was optimal.

In the more dilute blood-dextrose-citrate mixtures it seems probable that dextrose has a dual role. A small amount of the added sugar supplies a substrate for the glycolytic enzymes of the erythrocytes. The chief portion of the dextrose solution acts merely as a nonelectrolyte diluent for the plasma. This is suggested by the experiment of Friedman and DeGowin,[9] the data of which are presented in the chart on page 318. All blood samples were made with a blood/diluent ratio of 1.5, with the same volume and concentration of sodium citrate in each. When the diluent lacked dextrose, that sugar was added to one of the pair in a final concentration of 0.3 per cent. In each the presence of dextrose appreciably inhibited spontaneous hemolysis. When the diluent was salt-poor human albumin, preservation was as good as when dextrose was the diluent, provided the minimal concentration of the sugar was present.

The transfusion survival of cells stored in dextrose-citrate mixtures with low and high blood/diluent ratios was studied by Mollison and Young.[27] They concluded that the dilute mixtures showed a clear-cut superiority in surviving transfusion. Loutit[33] confirmed these findings and explained the mechanism of better preservation as being due to the fact that in the diluted plasma the pH was lowered from the shift of chlorides from cells to plasma.

ADDITION OF SODIUM CHLORIDE

Nearly all investigators have found the addition of sodium



chloride to be deleterious to the preservation of blood. Rous and Turner[1] noted increased hemolysis with the addition of Locke's

solution. DeGowin, Harris, and Plass[5,6] found that the mixture of sodium chloride and sodium citrate produced more hemolysis during storage than occurred in citrated blood alone. Reference to the figure on page 318 will show that when sodium chloride was added to any of the blood mixtures hemolysis was accelerated. The transfusion survival studies of Bushby, Kekwick, Marriott, and Whitby[22] showed that red cells stored in sodium chloride were rapidly lost from the circulation of the recipient. This was confirmed by Mollison and Young.[27]

The exact mechanism of the noxious action of sodium chloride in stored blood is unknown. Maizels and Whittaker[26] showed that during the second or third week of storage the sodium of the plasma permeates the erythrocytes to replace the lost potassium. Increasing the concentration of the plasma sodium may accelerate this process.

Addition of Dextrose

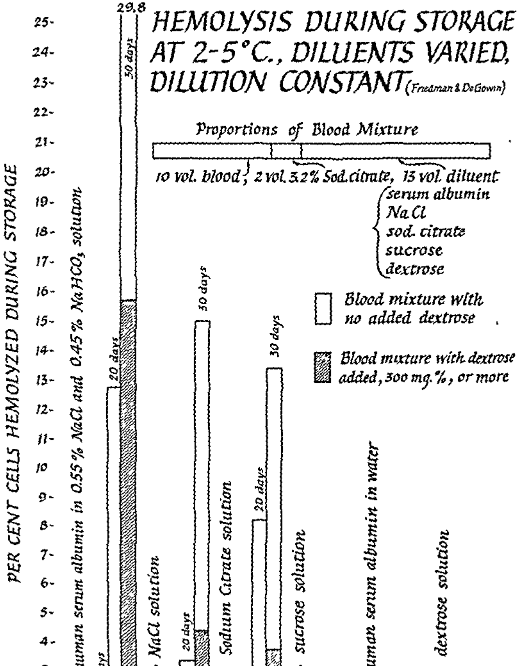
Influence on Spontaneous Hemolysis. The striking inhibition of hemolysis in stored blood to which dextrose had been added was first observed by Rous and Turner.[1] In some of the first quantitative studies of this effect DeGowin, Harris, and Plass[5,6] found that hemolysis in a modified Rous-Turner solution which contained dextrose was only 2 to 4 per cent as much as in citrated blood stored for thirty days at 5° C. These estimates were later confirmed by DeGowin *et al.*[7] by more precise methods.

Minimal Concentration of Dextrose. It is commonly stated that the optimal final concentration of dextrose in the blood preservative mixture is between 0.25 and 0.50 per cent. If the factor of dilution is excluded, this amount of sugar seems to provide maximum inhibition of hemolysis during storage. From certain experiments DeGowin, Harris, and Bell[7] concluded that a concentration of 3 per cent was optimal, but later studies by Friedman and DeGowin[9] showed that the factor of dilution had not been given due weight and that optimal dextrose concentration is probably less than 0.5 per cent. In the later studies it was found that the actual amount of dextrose which disappeared from the blood-dextrose-citrate mixtures stored at 2° C. for thirty days ranged from 73 to 140 mg. per 100 ml. of whole blood. This is considerably less than is usually added to preservative mixtures, so that 0.5 per cent concentration of dextrose represents a comfortable excess.

Mechanism of Inhibition of Hemolysis by Dextrose. As has been stated in considering the factor of dilution, it seems probable that dextrose solution plays a dual role in the preservation of

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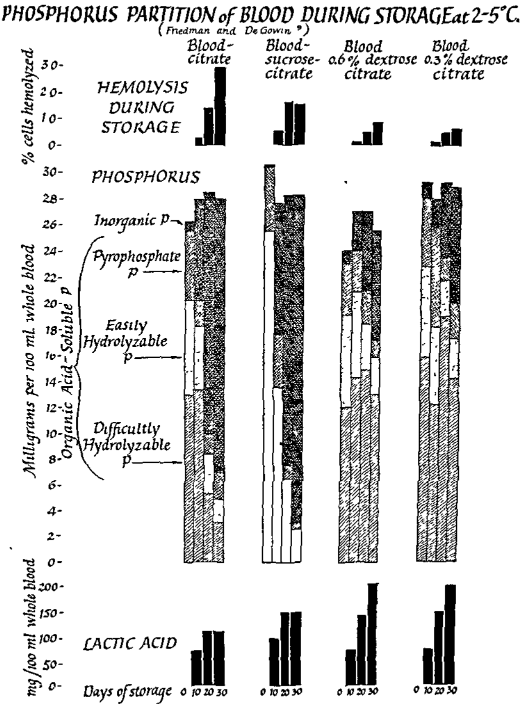
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increases when glycolysis in stored blood ceases. The details of the mechanism have not been elucidated except that the parallelism seems to bear a causal relationship.

Influence of Dextrose on Transfusion Survival. Practically all

studies on transfusion survival have shown that blood preserved in dextrose solutions are superior to those which are not.

Influence on Glycolysis. In the chart on page 320 the values are presented for the partition of the phosphate fractions in four blood mixtures stored for thirty days at 2° C. Two mixtures contained added dextrose, a third was diluted with sucrose, and the fourth was citrated blood. Lactic acid was formed in all four only until the dextrose was exhausted. There is an approximate relationship between the amount of inorganic phosphorus formed from the acid-soluble phosphorus and the rate of spontaneous hemolysis during storage. This has been confirmed by Rapoport.[44]

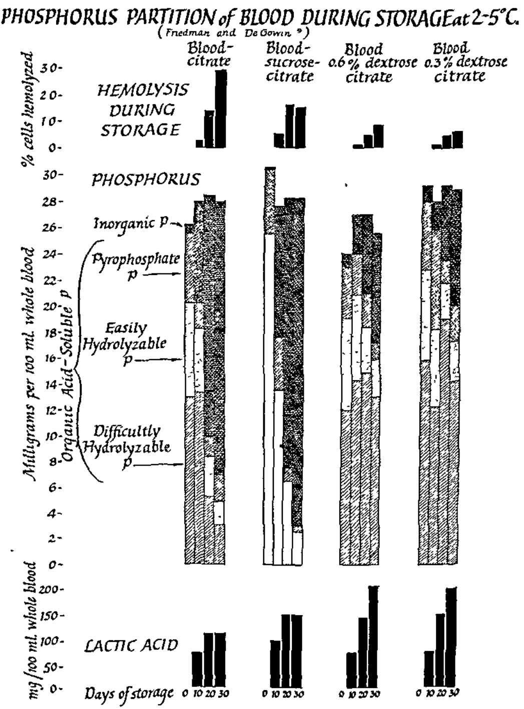
Influence on Osmotic Fragility. The osmotic fragility of the erythrocytes, as measured by the concentration of hypotonic sodium chloride solution in which hemolysis occurs, is an indication of the volume of the red cells. As more water diffuses into the erythrocyte, the volume increases and it is ruptured in higher concentrations of sodium chloride solution.

The addition of dextrose to blood-citrate mixtures introduces many complications in problems of osmotic relationships. A 5.4 per cent solution of dextrose has the same freezing point as that of blood plasma and it is therefore considered as a concentration isotonic with blood. It is not, however, isosmotic for erythrocytes because the sugar diffuses into the cells in varying amounts depending upon certain conditions such as temperature and the concentration of substances in the plasma.

The osmotic changes in blood cells during storage at 2° C. in various solutions were studied by DeGowin, Harris, Bell, and Hardin.[8] Table XXIII contains the protocols of three representative experiments with blood-citrate, blood-dextrose-citrate, and blood-sucrose-citrate. All solutions were isotonic, with the exception of sucrose which was used in a 4.5 per cent concentration (it was found that cells shrank in an isotonic solution of 10.3 per cent). In all three mixtures there is fair correlation between the changes in total cell volume (hematocrit) and the osmotic fragility of the *unwashed* cells, in blood-citrate and blood-dextrose-citrate. There is also a rough correspondence between the increase in osmotic fragility of the *washed* erythrocytes and the accumulation of inorganic phosphate described on page 307. But hemolysis occurred in sucrose-citrate mixtures when the cells were shrunken.

The difference between the osmotic fragility of unwashed and washed erythrocytes is great only in the blood mixtures which contain added dextrose. This is explained by the diffusion of dextrose and water into the erythrocytes within a few minutes after the sugar is added, and days before potassium has been lost from

blood. In addition to serving as a nonelectrolyte diluent, it serves as a substrate for glycolytic enzymes. It can be shown that hemolysis



increases when glycolysis in stored blood ceases. The details of the mechanism have not been elucidated except that the parallelism seems to bear a causal relationship.

Influence of Dextrose on Transfusion Survival. Practically all

at 27° C. The osmotic fragility of the cells increased as time passed. Initial hemolysis occurred in sodium chloride solutions as follows: Two minutes after mixture, 0.40 per cent NaCl; sixty minutes, 0.76 per cent NaCl; 120 minutes, 0.84 per cent; and 210 minutes, 0.88 per cent. When specimens were chilled after being at 27° C. for 120 minutes or more, they hemolyzed in the refrigerator, whereas controls at 27° C. did not. Apparently rapid chilling of erythrocytes after the addition of dextrose prevents the rapid diffusion of dextrose and water into the cells and consequent osmotic hemolysis. For a quantitative treatment of this subject the reader should consult Parpart *et al.*[43] This mechanism is particularly operative in a mixture where the plasma is made relatively hypotonic by the addition of a large amount of dextrose solution but it also applies to a lesser degree in blood-dextrose-citrate solutions of lesser volume. Because of this, the prechilling of preservative mixtures before the addition of blood is advised.

Hypertonic Dextrose Solutions. DeGowin, Harris, Bell, and Hardin[8] stored blood in the following mixture: Ten volumes of blood, two volumes of 3.2 per cent trisodium citrate (dihydric), and thirteen volumes of 10 per cent dextrose. The erythrocytes did not swell as much as in a mixture of similar proportions containing 5.4 per cent dextrose. Hemolysis did not occur when the blood was added to the solution which was not prechilled. Spontaneous hemolysis during storage was minimal. However, the osmotic fragility of the unwashed erythrocytes was extremely high, hemolysis occurred in 0.96 to 1.00 per cent sodium chloride solutions. The osmotic fragility of the washed cells was normal, showing that the cells were swollen because of the diffusion into them of dextrose and water. The blood mixture was transfused into a number of recipients with immediate extensive intravascular hemolysis.. Although the high dextrose content of the cells could be washed out in the test tube without rupturing the cells, under the conditions of transfusion the dextrose diffusion was not sufficiently rapid to avoid cell rupture.

Pretransfusion Test for Osmotic Fragility. Because of the variability of individual bloods during storage and the possibility of osmotic rupture in the recipient after transfusion, no stored or preserved blood should be transfused before a rapid test for osmotic fragility is performed upon it (p. 198).

Influence on Potassium Diffusion. The addition of dextrose was found by DeGowin, Harris, and Plass[10] to exert no influence on the loss of potassium from the erythrocytes during storage. This was confirmed by Aylward, Mainwaring, and Wilkinson,[35] and Rapoport.[44]

TABLE XXIII

Protocols of Representative Experiments on Osmotic Fragility of Erythrocytes During Storage in Various Mixtures for 30 Days at 2° C.[7,8]

Storage Mixture	Days of Storage	Spontaneous Hemolysis (% Cells Destroyed)	Changes in Total Cell Volume (%)	Osmotic Fragility Beginning—Complete Hemolysis (% NaCl)	
				Unwashed Cells	Washed Cells*
		Blood-Citrate			
23 Volumes of Blood + 2 Volumes of 3.2% Trisodium Dihydric Citrate	0	0	0	0.40–0.32	0.40–0.32
	5	0.09	0	0.64–0.32	0.64–0.36
	10				
	15	1.51	+13.8	0.64–0.32	0.64–0.36
	20	3.58	+21.4	0.88–0.40	0.88–0.40
	25	7.20	+31.5	0.88–0.40	0.88–0.40
	30	13.80	+47.0	0.92–0.44	0.92–0.44
		Blood-Dextrose-Citrate			
10 Volumes of Blood + 2 Volumes of 3.2% Sodium Citrate + 13 Volumes 5.4% Dextrose	0	0	+53.0	0.80–0.64	0.44–0.32
	5	0.03	+34.1	0.76–0.56	0.36–0.24
	10	0.08	+38.2	0.76–0.56	0.36–0.24
	15	0.09	+41.0	0.80–0.60	0.36–0.24
	20		+43.0	0.84–0.60	0.36–0.24
	25	0.21		0.80–0.64	0.40–0.28
	30	0.27	+50.0	0.84–0.64	0.36–0.28
		Blood-Sucrose-Citrate			
10 Volumes of Blood + 2 Volumes of 3.2% Sodium Citrate + 13 Volumes 4.5% Sucrose	0	0	+21.4	0.40–0.28	0.40–0.32
	5	0.03	−4.5	0.36–0.24	0.40–0.24
	10				
	15	1.14	−5.9	0.44–0.24	0.48–0.24
	20	1.01	−9.2	0.52–0.24	0.52–0.24
	25				
	30	2.57	−5.2	0.56–0.24	0.64–0.24

* Cells washed twice in 0.95% sodium chloride solution before being tested for osmotic fragility.

the cells. The increased fragility of cells in blood-citrate, on the contrary, is not reversible and must be explained by the accumulation of nondiffusible metabolites in the cells. This subject has also been considered by others.[37,43,44]

Effects of Temperature on Dextrose Diffusion. When 500 ml. of blood is added to the modified Rous-Turner solution which has been at room temperature, extensive hemolysis frequently occurs a few hours after the mixture is placed in the refrigerator.[6] This type of hemolysis can be prevented if the solution is chilled before the blood is added. The following experiment was performed.[8] Blood was added to the modified Rous-Turner mixture which was kept

tions are autoclaved in a basic medium the sugar is caramelized. Many workers have injected caramelized solutions intravenously without untoward effects but there is a widespread prejudice against the use of colored solutions in parenteral therapy.

Loutit, Mollison, and Young[11] tested a number of dextrose-citrate solutions to which various amounts of citric acid had been added so that the range of pH after autoclaving was 4.64 to 5.86. One solution was also studied in which disodium monohydric citrate was employed instead of trisodium citrate and citric acid. Blood stored in many of these solutions was found to undergo less spontaneous hemolysis during storage than in the mixture of the British Research Council, consisting of 420 ml. of blood, 100 ml. of 3 per cent dihydric trisodium citrate, and 20 ml. of 15 per cent dextrose solution. Transfusion studies showed that erythrocytes stored in solutions 2 and 8 (p. 334) survived slightly better than those in the Rous-Turner solution, which had been the best until that time. In the acid solutions the pH of the blood mixtures ranged from 7.1 to 6.8 initially. During storage for fourteen days, the pH fell to 6.85 to 6.5. The leakage of potassium from the erythrocytes seemed to be somewhat retarded although the rate of formation of inorganic phosphorus was somewhat increased. There was no evidence of toxicity in transfusions.

In a continuation of the study Loutit and Mollison[3] found that there were certain disadvantages to the dextrose-trisodium-citrate-citric-acid mixtures. Some, which were otherwise satisfactory, produced caramelization. Others, which were colorless after autoclaving, contained insufficient amounts of the citrate ion so that clotting occurred unless meticulous care was taken in the collection of blood. The preserving qualities of the dextrose-disodium-citrate mixture were further investigated. This did not caramelize during heating and spontaneous hemolysis during storage was inhibited satisfactorily. The erythrocytes in this solution survived transfusion as well as those in the Rous-Turner mixture. The authors cautioned that clots may form unless particular care is taken in the collection of the blood.

Later Loutit[33] presented data from more extensive studies on the effects of the pH of the blood mixture on the survival of erythrocytes during storage and after transfusion. The earlier conclusion was confirmed that acidification of the blood mixture aided preservation. The optimal initial plasma pH is probably 7.1 to 7.3. When the initial pH of the plasma is less than 7.0 preservation is not so satisfactory, although it is still somewhat superior to trisodium-citrate-dextrose mixtures. Blood in disodium citrate solution is preserved as well as in a mixture of trisodium citrate and dextrose,

Influence on Mechanical Fragility. In certain preservative mixtures to which dextrose was added DeGowin *et al.*[6] found that mechanical fragility of the red cells was better than without the sugar. The methods were inexact, however, and the effect of dilution could not be entirely excluded.

Influence on Granulocytes and Platelets. The addition of dextrose to blood-citrate mixtures has not been observed to prolong the life of leukocytes or platelets. Studies on this point are fragmentary.

Effect on Complement and Prothrombin. The addition of dextrose does not influence the rate at which complement and prothrombin deteriorate during storage.

Addition of Sucrose

The effect of the addition of sucrose to citrated blood was studied by Rous and Turner.[1] They found that it apparently inhibited spontaneous hemolysis during storage. DeGowin *et al.*[7] reported some inhibition of hemolysis from this sugar. Later Friedman and DeGowin[9] found that in the preparation of sucrose solutions by autoclaving some hydrolysis of the sugar to dextrose frequently occurs. When the solutions of sucrose were sterilized without heating, the preservative effect was much less (see chart on p. 320).

An isotonic solution of sucrose has a concentration of approximately 10.3 per cent. When a large volume is added to citrated blood, the cells shrink greatly, although spontaneous hemolysis proceeds. Some of this dehydrating effect on the cells occurs when a 4.5 per cent solution is employed (Table XXIII). Sucrose does not inhibit the glycolytic activity of the erythrocytes (see chart on p. 320).

Mollison and Young[27] found that erythrocytes stored in a sucrose-citrate mixture survived transfusion very poorly, although hemolysis during storage was little and osmotic fragility was less than normal.

Addition of Dextrin

In their early experiments Rous and Turner[1] tried the addition of dextrin to citrated blood and concluded that it had virtue in preserving dog blood. Maizels and Whittaker[36] studied a dextrin-citrate mixture which inhibited hemolysis during storage. Cells in this solution survived transfusion fairly well.[37] Dextrin has no superiority over dextrose, however, and has not been employed to any great extent.

Varying the pH of Dextrose-Citrate Solutions

The interest in the pH of dextrose-citrate mixtures arose from the fact that trisodium citrate is alkaline and when dextrose solu-

produce blood mixtures which can be administered directly from the collection bottle into the vein of the recipient through flask and line filters.

The outdating periods of various blood mixtures are always arbitrary and empiric. Often they have been established by the rate of spontaneous hemolysis during storage. When supported by data from transfusion survival studies, the period of safe storage is still an arbitrary standard. The operator of a blood bank should be cautioned that there are individual differences in blood and that in any preservative mixture certain bloods will show more rapid deterioration than others. Regardless of the preservative solution employed or the time of storage *the rapid osmotic fragility test (p. 198) should be mandatory before transfusion of dextrose-citrate mixtures.* Outdating periods can only be considered as average estimates.

Rous-Turner Solution[1]

Composition.

Blood	500 ml.
3.8% trisodium citrate (2 $Na_3C_6H_5O_7$ + 11 H_2O) in water	233 ml.
5.4% anhydrous dextrose in water	833 ml.
Total volume	*1566 ml.*
Blood/diluent ratio	*2.1*

Preparation. Autoclave the citrate and dextrose solutions separately at 121° C. (250° F.) for twenty minutes. Then mix the solutions together aseptically, when cool. The solution must be cold when the blood is added. The supernatant plasma is discarded just before use and the erythrocytes are suspended in 0.9 per cent sodium chloride solution and transfused.

Spontaneous Hemolysis. This solution probably inhibits hemolysis during storage better than any other so far devised. It is used as the standard of comparison for other blood mixtures.

Transfusion Survival. Adequate studies of the survival in the recipient of erythrocytes preserved in this solution are available. They show that this mixture preserved cells as well as any so far studied.

Clinical Experience. So far as is known, this solution has been used but little clinically.

Average Outdating Period. About twenty-eight to thirty days, as based on the degree of spontaneous hemolysis during storage. Studies after transfusion show that the erythrocytes stored for fourteen days survive as well as when fresh blood is injected.

Mechanical Difficulties in Transfusion. The great dilution and the high concentration of sodium citrate prevent much clotting with ordinary handling. The filtration rate is good.

but the addition of the sugar to the disodium citrate solution improves preservation. The preservative effect of dilution in *trisodium-citrate-dextrose mixtures* was considered as probably caused by the hydrogen and other penetrating ions diffusing from the cells into the dilute plasma, making the latter slightly more acid. Dilution of the acidified mixtures does not improve preservation.

ADDITION OF PHOSPHATE BUFFERS

Muether and Andrews[39] adjusted the pH of the modified Rous-Turner solution[5,6] by buffering with monobasic and dibasic sodium phosphate so that the dextrose and citrate could be heated together without caramelizing. The preservation of erythrocytes in this solution has been satisfactory. Denstedt *et al.*[28] reduced the volume of dextrose in the mixture and buffered it with monobasic sodium phosphate and sodium hydroxide. Extensive transfusion survival studies showed that the buffered-mixtures preserved erythrocytes slightly better than did the nonbuffered dextrose-citrate mixture. Parpart *et al.*[43] confirmed the beneficial effect of phosphate buffers.

BLOOD PRESERVATIVE MIXTURES

In this section is presented some of the more frequently used blood preservative mixtures, with critical analysis whenever possible. Such a presentation can only result in imperfect evaluations for many reasons. The formulas of the solutions have been changed from time to time by the authors or commercial firms without formal notice in the literature. The data for the survival of erythrocytes *in vitro* and *in vivo* are frequently inadequate or are not comparable with those of other mixtures. The amount of clinical experience with some mixtures can only be guessed. The statements regarding the advantages and disadvantages can only be the considered opinions of the authors of this book, who cannot claim personal experience with all the solutions mentioned.

The mechanical difficulty experienced during transfusion with a blood mixture is extremely important, but frequently ignored in the scientific literature. It should be obvious that no matter how well the blood is preserved *it is useless unless it is injectable into the veins of the recipient in time to save his life.* The mechanical difficulty encountered during transfusion varies greatly with the operator and the apparatus employed. In discussing filterability of the blood mixture no consideration will be given to the procedure in which the blood is poured through an open filter just before transfusion. Although this is a practical and safe method, the goal should be to

children, or adults with increased blood volume. (2) Two sterile solutions must be mixed aseptically in preparation.

Alternate Formula (DeGowin).

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$)	3	gm
Citric acid ($C_3H_4OH(COOH)_3 + H_2O$)	0.46	gm.
Anhydrous dextrose	32 6	gm.
Water	700	ml.
Total volume	*1200 ml.*	
Blood/diluent ratio	*1.4*	

Preparation. Add the sodium citrate, citric acid, and dextrose to the water, dissolve, and autoclave at 121° C. (250° F.) for twenty minutes. The solution must be cold when the blood is added. The mixture is transfused without partition of the cells and plasma.

Comments. This solution has the advantage over the preceding formula that all the ingredients are autoclaved together without caramelization.

BUFFERED ROUS-TURNER SOLUTION (Muether and Andrews[19])

Composition.

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$)	3.2	gm.
Anhydrous dextrose	35.1	gm.
Monobasic sodium phosphate ($NaH_2PO_4 + H_2O$)	0.1875	gm.
Dibasic sodium phosphate (Na_2HPO_4)	1.875	gm.
Water	750	ml.
Total volume	*1250 ml.*	
Blood/diluent ratio	*1 5*	

Preparation. Dissolve the citrate and phosphates in a small amount of water. Make a separate solution of the dextrose and add the buffer mixture, finally making the solution up to the required volume. Autoclave at 121° C. (250° F.) for twenty minutes. The solution must be cold when the blood is added. The mixture is transfused without partition of cells and plasma.

Spontaneous Hemolysis. Hemolysis during storage is inhibited as well as in the other modifications of the Rous-Turner mixture.

Transfusion Survival. Inadequate studies are available on transfusion survival of erythrocytes stored in this solution.

Clinical Experience. This solution has been in use for many years in several transfusion services and has been commercially available in the United States. The results have been satisfactory.

Average Outdating Period. Between twenty-one and thirty days. The upper limit has been determined by the rate of spontaneous hemolysis during storage.

Comments. *Advantages:* (1) Excellent preservation of erythrocytes *in vitro* and *in vivo.* (2) Especially useful in the transfusion of red cell suspensions in the treatment of chronic anemias. *Disadvantages:* (1) With the classic formula, the dextrose and citrate solutions must be prepared and autoclaved separately. (2) The concentration of citrate in the plasma is considered so high that the plasma is discarded, although proof of the toxicity of this amount of citrate has not been forthcoming (p. 296). (3) If the entire blood mixture is transfused, the bulk is too great for the circulation of some patients.

MODIFIED ROUS-TURNER SOLUTION (DeGowin, Harris, and Plass[5,6])

Composition.

Blood	500 ml.
3.2% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	100 ml.
5 4% anhydrous dextrose in water	650 ml.
Total volume	*1250 ml*
Blood/diluent ratio	*1.5*

Preparation. Autoclave the citrate and dextrose solutions separately at 121° C. (250° F.) for twenty minutes. Mix the solutions aseptically when cool. The solution must be cold when the blood is added. The mixture is transfused without partition of cells and plasma.

Spontaneous Hemolysis. Hemolysis during storage is inhibited as well as in the original Rous-Turner solution.

Transfusion Survival. Very few studies have been made of transfusion survival of cells stored in this mixture.

Clinical Experience. This solution has been employed for many years in several transfusion services with satisfactory results.

Average Outdating Period. Between twenty-one and thirty days. The upper limit is permitted by the degree of spontaneous hemolysis during storage but many bloods are rejected for transfusion after twenty-one days because of increased osmotic fragility of the erythrocytes (p. 321).

Mechanical Difficulties in Transfusion. There is little clotting during storage or collection. The filterability is good.

Comments. *Advantages:* Excellent preservation *in vitro* and apparently good survival *in vivo* as judged by clinical observations. (2) Minimal mechanical difficulty in transfusion. (3) The bulk, made up largely of dextrose solution, is desirable in patients who are dehydrated or in hemorrhagic shock. (4) Excellent yield of dilute plasma by the sedimentation method. The supernatant plasma contains a minimum of free hemoglobin at the outdating period. *Disadvantages:* (1) The bulk is not desired in transfusions to young

children, or adults with increased blood volume. (2) Two sterile solutions must be mixed aseptically in preparation.

Alternate Formula (DeGowin).

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2H_2O$)	3	gm.
Citric acid ($C_3H_4OH(COOH)_3 + H_2O$)	0 46	gm.
Anhydrous dextrose	32 6	gm.
Water	700	ml.
Total volume	*1200 ml.*	
Blood/diluent ratio	*1 1*	

Preparation. Add the sodium citrate, citric acid, and dextrose to the water, dissolve, and autoclave at 121° C. (250° F.) for twenty minutes. The solution must be cold when the blood is added. The mixture is transfused without partition of the cells and plasma.

Comments. This solution has the advantage over the preceding formula that all the ingredients are autoclaved together without caramelization.

BUFFERED ROUS-TURNER SOLUTION (Muether and Andrews[39])

Composition.

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2H_2O$)	3.2	gm
Anhydrous dextrose	35 1	gm.
Monobasic sodium phosphate ($NaH_2PO_4 + H_2O$)	0.1875	gm.
Dibasic sodium phosphate (Na_2HPO_4)	1.875	gm.
Water	750	ml.
Total volume	*1250 ml.*	
Blood/diluent ratio	*1 5*	

Preparation. Dissolve the citrate and phosphates in a small amount of water. Make a separate solution of the dextrose and add the buffer mixture, finally making the solution up to the required volume. Autoclave at 121° C. (250° F.) for twenty minutes. The solution must be cold when the blood is added. The mixture is transfused without partition of cells and plasma.

Spontaneous Hemolysis. Hemolysis during storage is inhibited as well as in the other modifications of the Rous-Turner mixture.

Transfusion Survival. Inadequate studies are available on transfusion survival of erythrocytes stored in this solution.

Clinical Experience. This solution has been in use for many years in several transfusion services and has been commercially available in the United States. The results have been satisfactory.

Average Outdating Period. Between twenty-one and thirty days. The upper limit has been determined by the rate of spontaneous hemolysis during storage.

Comments. *Advantages:* (1) Excellent preservation of erythrocytes *in vitro* and *in vivo*. (2) Especially useful in the transfusion of red cell suspensions in the treatment of chronic anemias. *Disadvantages:* (1) With the classic formula, the dextrose and citrate solutions must be prepared and autoclaved separately. (2) The concentration of citrate in the plasma is considered so high that the plasma is discarded, although proof of the toxicity of this amount of citrate has not been forthcoming (p. 296). (3) If the entire blood mixture is transfused, the bulk is too great for the circulation of some patients.

MODIFIED ROUS-TURNER SOLUTION (DeGowin, Harris, and Plass[5,6])

Composition.

Blood	500 ml.
3 2% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	100 ml.
5 4% anhydrous dextrose in water	650 ml.
Total volume	*1250 ml.*
Blood/diluent ratio	*1.5*

Preparation. Autoclave the citrate and dextrose solutions separately at 121° C. (250° F.) for twenty minutes. Mix the solutions aseptically when cool. The solution must be cold when the blood is added. The mixture is transfused without partition of cells and plasma.

Spontaneous Hemolysis. Hemolysis during storage is inhibited as well as in the original Rous-Turner solution.

Transfusion Survival. Very few studies have been made of transfusion survival of cells stored in this mixture.

Clinical Experience. This solution has been employed for many years in several transfusion services with satisfactory results.

Average Outdating Period. Between twenty-one and thirty days. The upper limit is permitted by the degree of spontaneous hemolysis during storage but many bloods are rejected for transfusion after twenty-one days because of increased osmotic fragility of the erythrocytes (p. 321).

Mechanical Difficulties in Transfusion. There is little clotting during storage or collection. The filterability is good.

Comments. *Advantages:* Excellent preservation *in vitro* and apparently good survival *in vivo* as judged by clinical observations. (2) Minimal mechanical difficulty in transfusion. (3) The bulk, made up largely of dextrose solution, is desirable in patients who are dehydrated or in hemorrhagic shock. (4) Excellent yield of dilute plasma by the sedimentation method. The supernatant plasma contains a minimum of free hemoglobin at the outdating period. *Disadvantages:* (1) The bulk is not desired in transfusions to young

Comment. Designed to furnish a good yield of clear plasma by sedimentation after the whole blood has been kept in the bank. *Advantages:* (1) This solution produces an excellent dilute plasma by the sedimentation method after storage for sixteen days. (2) The mechanical difficulties in transfusion are infrequent. (3) The yield of plasma by sedimentation is greater than in the Rous-Turner type of solution because of less swelling of the erythrocytes. (4) The added electrolyte and dextrose is desirable in the treatment of patients with certain types of dehydration. *Disadvantages:* (1) The bulk might be contraindicated in transfusing young children, or adults with increased plasma volume. (2) The rate of spontaneous hemolysis during storage is somewhat greater than in some other solutions.

McGill Solution I

(Denstedt, Osborne, Stansfield, and Rochlin[23])

Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
3.2% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	100 ml.
5.4% anhydrous dextrose in water	150 ml.
Total volume	*750 ml.*
Blood/diluent ratio	0.5

Preparation. Make up the citrate and dextrose solutions and autoclave them separately at 121° C. (250° F.) for twenty minutes. Mix the sterile solutions together aseptically when cool. The solution should be cold when the blood is added.

Spontaneous Hemolysis. The rate of hemolysis during storage is probably somewhat greater than in the more dilute Rous-Turner type of solution, but the authors report less than one per cent of erythrocytes hemolyzed in storage from forty-two to sixty days.

Transfusion Survival. There are adequate studies by the authors which show that the survival of erythrocytes is unimpaired up to eighteen days of storage. Storage for twenty-five to thirty days gave good survival rates.

Clinical Experience. The solution has had some clinical use in Canada and the United States but the extent is unknown.

Average Outdating Period. Between twenty-five and thirty days, based on transfusion survival studies.

Mechanical Difficulties in Transfusion. Because of the dilution of the plasma clotting during blood collection or storage is not great. The filterability of the blood mixture is good.

Comments. *Advantages:* (1) Good preservation as judged by tests *in vitro* and *in vivo*. (2) The bulk should not be a contraindication in most transfusions. (3) The yield of dilute plasma from

Mechanical Difficulties in Transfusion. With the dilution of plasma there is little clotting during collection or storage of the blood. The blood mixture filters well.

Comments. *Advantages:* (1) Excellent preservation of the erythrocytes *in vitro* and clinically good results from transfusion. (2) Minimal mechanical difficulties in transfusion. (3) The bulk is desirable in patients who are dehydrated from hemorrhagic shock or other causes. (4) There is an excellent yield of dilute plasma by the sedimentation method. The plasma contains a small concentration of free hemoglobin at the outdating period. (5) All the ingredients of the solution are autoclaved together. *Disadvantages:* (1) The bulk is undesirable in the transfusion of young children, or adults with increased plasma volume. (2) The formula contains a multiplicity of chemicals. (3) In some patients the added phosphate might be considered to be contraindicated.

DEXTROSE-SALINE-CITRATE SOLUTION (Alsever and Ainslie[40])

Composition.

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$)	4	gm.
Citric acid* ($Na_3H_4OH\ (COOH)_3 + H_2O$)	0.25	gm.
Anhydrous dextrose	9.33	gm.
Sodium chloride	2.09	gm.
Water	500	ml.
Total volume	*1000 ml.*	
Blood/diluent ratio	*1.0*	

*This was later added by Dr. Alsever.

Preparation. Put the ingredients into solution and autoclave at 121° C. (250° F.) for twenty minutes. The mixture should be cold when the blood is added.

Spontaneous Hemolysis. Hemolysis occurs during storage at a somewhat faster rate than in solutions of the Rous-Turner type, presumably because of the added sodium chloride.

Transfusion Survival. Inadequate studies are available to form a conclusion as to the survival of erythrocytes during transfusion.

Clinical Experience. This solution has been widely used in the United States and has been commercially available. It was used to some extent in the United States Army during World War II. The results have been satisfactory.

Average Outdating Period. Between sixteen and twenty-one days. The upper limit was determined chiefly by the rate of spontaneous hemolysis during storage.

Mechanical Difficulties in Transfusion. There is little clotting during blood collection or storage. The dilute plasma permits good [illegible]ation of the blood mixture.

Comment. Designed to furnish a good yield of clear plasma by sedimentation after the whole blood has been kept in the bank. *Advantages:* (1) This solution produces an excellent dilute plasma by the sedimentation method after storage for sixteen days. (2) The mechanical difficulties in transfusion are infrequent. (3) The yield of plasma by sedimentation is greater than in the Rous-Turner type of solution because of less swelling of the erythrocytes. (4) The added electrolyte and dextrose is desirable in the treatment of patients with certain types of dehydration. *Disadvantages:* (1) The bulk might be contraindicated in transfusing young children, or adults with increased plasma volume. (2) The rate of spontaneous hemolysis during storage is somewhat greater than in some other solutions.

McGill Solution I

(Denstedt, Osborne, Stansfield, and Rochlin[11])

Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
3.2% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	100 ml.
5.4% anhydrous dextrose in water	150 ml.
Total volume	*750 ml.*
Blood/diluent ratio	*0 5*

Preparation. Make up the citrate and dextrose solutions and autoclave them separately at 121° C. (250° F.) for twenty minutes. Mix the sterile solutions together aseptically when cool. The solution should be cold when the blood is added.

Spontaneous Hemolysis. The rate of hemolysis during storage is probably somewhat greater than in the more dilute Rous-Turner type of solution, but the authors report less than one per cent of erythrocytes hemolyzed in storage from forty-two to sixty days.

Transfusion Survival. There are adequate studies by the authors which show that the survival of erythrocytes is unimpaired up to eighteen days of storage. Storage for twenty-five to thirty days gave good survival rates.

Clinical Experience. The solution has had some clinical use in Canada and the United States but the extent is unknown.

Average Outdating Period. Between twenty-five and thirty days, based on transfusion survival studies.

Mechanical Difficulties in Transfusion. Because of the dilution of the plasma clotting during blood collection or storage is not great. The filterability of the blood mixture is good.

Comments. *Advantages*: (1) Good preservation as judged by tests *in vitro* and *in vivo.* (2) The bulk should not be a contraindication in most transfusions. (3) The yield of dilute plasma from

outdated blood by sedimentation is good. *Disadvantages*: The preparation requires the combination of two sterile solutions under aseptic conditions.

McGill Solution II

(Denstedt, Osborne, Stansfield, and Rochlin[28])

Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
3.2% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	100 ml.
Isotonic buffer solution	50 ml.
5.4% anhydrous dextrose in water	100 ml.
Total volume	*750 ml.*
Blood/diluent ratio	*0.5*

Preparation. Prepare the buffer solution, the citrate solution, and the dextrose solution separately. The composition of the isotonic phosphate buffer solution is as follows:

4.14% (0 3 molar) monobasic sodium phosphate ($NaH_2PO_4+H_2O$)	1000 ml.
1 2% (0 3 molar) sodium hydroxide (NaOH)	925 ml.
Water	480 ml.

Autoclave the three solutions separately at 121° C. (250° F.) for twenty minutes. After cooling combine the three sterile solutions aseptically. The solution should be cold before the blood is added.

Comment. This solution possesses a slight advantage in transfusion survival over the unbuffered solution by the same authors. It is, however, more complicated to prepare. The concentration of phosphate ion might be considered contraindicated in some patients.

Medical Research Council (British) Solution[41]

Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
3% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	120 ml.
15% anhydrous dextrose in water	24 ml.
Total volume	*644 ml.*
Blood/diluent ratio	*0 28*

Alternative Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
3% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	120 ml
30% anhydrous dextrose in water	12 ml
Total volume	*632 ml.*
Blood/diluent ratio	*0.26*

Preparation. Prepare the citrate and dextrose solutions and autoclave them separately at 121° C. (250° F.) for twenty minutes.

The blood is collected in the citrate solution and the dextrose solution is added afterward. If the blood mixture has cooled, the dextrose solution should be cool when added.

Spontaneous Hemolysis. With storage for twenty-one days the hemolysis is about three times that in the Rous-Turner mixture,[14] and at twenty-eight days about 1.5 per cent of the erythrocytes have been destroyed.

Transfusion Survival. Adequate data on transfusion survival are available[14] and show that erythrocytes stored for fourteen days are less well preserved than in the Rous-Turner solution or the acid-citrate-dextrose mixture.

Clinical Experience. This preservative mixture was widely used by the War Emergency Transfusion Services in Great Britain during World War II for the treatment of civilian casualties and was apparently satisfactory.

Average Outdating Period. Between fourteen and twenty-one days, determined by hemolysis during storage and transfusion survival.

Mechanical Difficulties in Transfusion. As the plasma has relatively slight dilution the amount of clotting during collection and storage of blood might be expected to be greater than in the more dilute solutions. With prolonged storage there is apt to be a heavy precipitation of fibrin and globulin in the plasma. When fine mesh filters are employed more difficulty is encountered than in more dilute solutions.

Comments. *Advantages*: (1) Fair preservation of erythrocytes. (2) Compactness in transportation and administration. *Disadvantages*: (1) Requires the mixing of two sterile solutions aseptically. (2) The plasma is not sufficiently dilute to give a high yield from outdated blood by the sedimentation method. (3) Difficulty in passing fine mesh filters.

British Army Blood Transfusion Service Solution

Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
3% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$) in water	114 ml.
10% anhydrous dextrose in water	23 ml.
Total volume	*637 ml.*
Blood/diluent ratio	*0 27*

Preparation. Prepare the dextrose and citrate solutions and autoclave them separately at 121° C. (250° F.) for twenty minutes. The blood is collected in the citrate solution and the dextrose solution is later added aseptically while both solutions are cold.

Comment. This solution does not differ materially from those of the British Medical Research Council. It was widely used in the British Army Blood Transfusion Service during World War II.

Acid-Citrate-Dextrose Solutions (ACD)

It is impossible to list all the mixtures containing dextrose, sodium citrate, and citric acid which are now being used. The term ACD does not refer to any particular formula. In general the purpose has been to make a solution which can be autoclaved with all the ingredients without caramelization of the dextrose, which preserves erythrocytes well, and which produces only slight dilution of the plasma. Some American workers have attempted the use of solutions in which the dilution of plasma was less than originally described. The authors of this book do not recommend blood preservative mixtures with a blood diluent ratio of less than 0.25 because of the excessive clotting during collection and storage of the blood and the difficulty with filtration in a closed system of transfusion.

Composition of Solution 2 (Loutit, Mollison, and Young[38]). (Recalculated for 500 ml. of blood)

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$)	3 2	gm.
Citric acid ($C_6H_8O_7 + H_2O$)	0.36	gm
Anhydrous dextrose	3.5	gm.
Water	131	ml.

Total volume 631 *ml.*
Blood/diluent ratio 0 26

Composition of Solution 8 (Loutit, Mollison, and Young[38]). (Recalculated for 500 ml. of blood)

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$)	0.76	gm.
Citric acid ($C_6H_8O_7 + H_2O$)	0.43	gm.
Anhydrous dextrose	7	gm.
Water	143	ml.

Total volume 643 *ml.*
Blood/diluent ratio 0.28

Composition of an American Solution.

Blood	500	ml
Trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$)	1.66	gm.
Citric acid ($C_6H_8O_7 + H_2O$)	0.6	gm.
Anhydrous dextrose	3 7	gm.
Water	125	ml.

Total volume 625 *ml.*
Blood/diluent ratio 0.25

Composition of Another American Solution.

Blood	500	ml.
Trisodium citrate ($Na_3C_6H_5O_7 + 2\,H_2O$)	1.65	gm
Citric acid ($C_6H_8O_7 + H_2O$)	0.6	gm
Anhydrous dextrose	1.8	gm
Water	75	ml.

Total volume 575 *ml*
Blood/diluent ratio 0.15

Preparation. (All ACD solutions). The ingredients are added to water and the solution is autoclaved at 121° C. (250° F.) for twenty minutes. The solution should be cold when the blood is added. Meticulous attention must be given during blood collection to insure thorough and prompt mixing of the solution with the blood so that clotting is avoided.

Caramelization. Loutit *et al.*[14] reported that Solution No. 2 produced some caramelization during heating in the autoclave. They used vertical autoclaves, heated by gas, from which the air must be forced by a free flow of steam for some time before the pressure is permitted to build up. Undoubtedly this subjects the solutions to more heat than is the case in the use of steam-heated autoclaves from which the air is removed by suction. It has never been demonstrated that the intravenous injection of small amounts of caramelized dextrose solution is harmful.

Spontaneous Hemolysis. The degree of hemolysis during storage in the ACD solutions is slightly greater than in the Rous-Turner solution.

Transfusion Survival. Adequate studies[18] are available on Solulutions 2 and 8 to show that erythrocytes stored in them survive in the recipient as long, or slightly longer, than those preserved in the Rous-Turner mixture. Formulas of the American type have not received adequate tests in comparison.

Clinical Experience. It is not known how widely Solutions 2 and 8 have been used in Great Britain. They have not been employed to any extent in the United States. Formulas of the American types have been widely used in the United States, particularly with a volume of 625 ml.

Average Outdating Period. From twenty-one to thirty days. The upper limit is usually established by the amount of spontaneous hemolysis during storage.

Mechanical Difficulties in Transfusion. Because of the small dilution of plasma, clotting during blood collection and storage is frequent. Some fibrin formation during collection may be avoided by the use of the inverted vacuum bottle so that the blood enters the

container through a column of the preservative solution. The precipitation of fibrin sheets in the plasma during storage is unavoidable.

Comment. *Advantages:* (1) Excellent preservation of erythrocytes. (2) Compactness in transfusing young children, and in adults with increased plasma volume. (3) There is but a single solution to prepare. *Disadvantages:* (1) More difficulties in the mechanics of transfusion than with the dilute mixtures. (2) Poor yield of plasma from outdated blood by the sedimentation technique. (3) Some precipitation of fibrin in the plasma during storage. (4) Lack of additional water and dextrose which is desirable in the treatment of hemorrhagic shock or in other conditions accompanied by dehydration.

DISODIUM-CITRATE-DEXTROSE SOLUTION (Loutit and Mollison[3])

Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
Disodium citrate (monohydric)	2.33 gm.
Anhydrous dextrose	3 gm.
Water	143 ml.
Total volume	*643 ml*
Blood/diluent ratio	*0 28*

Preparation. Add the citrate and dextrose to the same solution and autoclave at 121° C. (250° F.) for twenty minutes. The solution should be cold when the blood is added.

Spontaneous Hemolysis. The rate of hemolysis during storage is but slightly faster than in the Rous-Turner solution.[38]

Transfusion Survival. Adequate studies are available to indicate that erythrocytes preserved in this solution from twenty to twenty-eight days survive transfusion nearly as well as fresh blood.[3]

Clinical Experience. This solution has been little used in the United States although considerable experience has been acquired with it in Great Britain.

Average Outdating Period. From twenty-five to thirty days, based on transfusion survival studies.

Mechanical Difficulties in Transfusion. The authors emphasize that special precautions are necessary during the collection of blood to avoid the formation of clots. It should also be realized that the British use much coarser blood filters than have been the fashion in the United States and it seems probable that more difficulty in transfusion would be encountered here from that fact.

Comment. *Advantages:* (1) Excellent preservation of erythrocytes *in vitro* and *in vivo.* (2) In the preparation of the solution there are only two chemicals to weigh and they are autoclaved in the same solution. (3) The solution is compact and hence suitable for trans-

fusion into children and adults with increased plasma volume. *Disadvantages:* (1) The mechanical difficulties in transfusion, inherent in other compact solutions, are admittedly present. (2) It lacks the volume of fluid which is required by the patient with hemorrhagic shock or dehydration. (3) The disodium salt of citric acid is not procurable in the United States in a pyrogen-free state.

Citrated Blood

Composition.

Blood	500 ml
3.2% trisodium citrate ($Na_3C_6H_5O_7 + 2\ H_2O$)	50 to 100 ml.
Total volume	*550 to 600 ml.*
Blood/diluent ratio	*0.1 to 0.2*

Preparation.[1] Autoclave the citrate solution at 121° C. (250° F.) for twenty minutes. The solution should be cold when the blood is added.

Spontaneous Hemolysis. Hemolysis during storage in citrated blood is greater than any other solution yet in use. The addition of even small amounts of dextrose retards deterioration.

Transfusion Survival. Erythrocytes stored in citrate solution alone survive transfusion very poorly.

Clinical Experience. The solution has been widely used in the United States for the storage of blood, particularly in the early days of blood banks. It is still most widely employed for the transfusion of fresh blood.

Average Outdating Period. From five to ten days. The upper limit is determined both by the amount of hemolysis and the poor transfusion survival.

Mechanical Difficulties in Transfusion. Clotting during collection and storage is frequent and troublesome because of the slight dilution of the plasma. Filtration is complicated for the same reason.

Comment. *Advantages:* (1) This mixture is preferred for transfusions of fresh blood. (2) Citrated blood can be used when plasma is to be prepared from it by centrifugation within twenty-four hours after collection. *Disadvantages:* (1) This solution is in no sense a blood preservative and has no merit in the storage of blood for transfusion. There is no excuse for the storage of citrated blood when the addition of 25 ml. of dextrose solution to 550 ml. of blood mixture will so markedly retard deterioration.

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container through a column of the preservative solution. The precipitation of fibrin sheets in the plasma during storage is unavoidable.

Comment. *Advantages:* (1) Excellent preservation of erythrocytes. (2) Compactness in transfusing young children, and in adults with increased plasma volume. (3) There is but a single solution to prepare. *Disadvantages:* (1) More difficulties in the mechanics of transfusion than with the dilute mixtures. (2) Poor yield of plasma from outdated blood by the sedimentation technique. (3) Some precipitation of fibrin in the plasma during storage. (4) Lack of additional water and dextrose which is desirable in the treatment of hemorrhagic shock or in other conditions accompanied by dehydration.

DISODIUM-CITRATE-DEXTROSE SOLUTION (Loutit and Mollison[3])

Composition (recalculated for 500 ml. of blood).

Blood	500 ml.
Disodium citrate (monohydric)	2.33 gm.
Anhydrous dextrose	3 gm.,
Water	143 ml.
Total volume	*643 ml*
Blood/diluent ratio	*0 28*

Preparation. Add the citrate and dextrose to the same solution and autoclave at 121° C. (250° F.) for twenty minutes. The solution should be cold when the blood is added.

Spontaneous Hemolysis. The rate of hemolysis during storage is but slightly faster than in the Rous-Turner solution.[38]

Transfusion Survival. Adequate studies are available to indicate that erythrocytes preserved in this solution from twenty to twenty-eight days survive transfusion nearly as well as fresh blood.[3]

Clinical Experience. This solution has been little used in the United States although considerable experience has been acquired with it in Great Britain.

Average Outdating Period. From twenty-five to thirty days, based on transfusion survival studies.

Mechanical Difficulties in Transfusion. The authors emphasize that special precautions are necessary during the collection of blood to avoid the formation of clots. It should also be realized that the British use much coarser blood filters than have been the fashion in the United States and it seems probable that more difficulty in transfusion would be encountered here from that fact.

Comment. *Advantages:* (1) Excellent preservation of erythrocytes *in vitro* and *in vivo.* (2) In the preparation of the solution there are only two chemicals to weigh and they are autoclaved in the same solution. (3) The solution is compact and hence suitable for trans-

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CHAPTER 14

Transportation of Whole Blood

By Robert C. Hardin

The transportation of blood and blood products is a natural outgrowth of blood banking. As in many related problems war medicine has stimulated endeavor in this field. The modern practice in warfare is to move medical facilities to the wounded rather than to subject a severely injured man to a long and often detrimental journey to the rear. This concept may well be developed in civilian practice in time of disaster. Under circumstances such as these the doctor must be equipped with all the paraphernalia of present day medicine among which is the material necessary in the treatment of shock. Of more importance in everyday life is the constant development, particularly in larger population centers, of central blood banks serving several hospitals within a certain geographical area. Even where there is no such organization, larger blood banks may be called upon to furnish an occasional bottle of blood of rare type or group to a smaller institution. Problems involved in transportation are therefore of concern to all.

Plasma, serum, and albumin solutions are relatively stable and unaffected by environmental factors with the exception of temperature low enough to cause freezing which may burst bottles. This presents no greater problem than is encountered in the handling of many other biologicals and is easy of solution. Whole blood possesses no such stability and requires special handling if it is to arrive at its destination in a usable state.

The feasibility of transportation of blood was first demonstrated in World War I shortly after the fundamental principles of preservation were discovered.[1] It was not attempted on a large scale, however, until the Loyalist forces made regular use of this kind of

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erythrocytes is not affected by transportation but by conditions of storage encountered during shipment.

Effects of Low Atmospheric Pressure. One rather theoretical consideration which arises in the shipment of blood by aircraft is the effect of the low atmospheric pressure at high altitude. Fortunately air transports do not fly sufficiently high to encounter extremely low pressures. The entire problem is solved by airtight, secure closure of bottles. This both stabilizes pressure within the container and prevents displacement of the cap.

PRESERVATION AND TRANSPORTATION

Preservative Solutions. The principles involved in the preservation of blood under conditions met in transportation do not differ from those governing it in ordinary practice. Therefore the content of the preservative mixture should not be altered (Chap. 13).

Refrigeration. It has been repeatedly demonstrated that the optimal temperature for the preservation of blood is between 2° and 10° C. However, the extra weight and space required by ice and equipment raises the question of whether one might not dispense with refrigeration, particularly during air shipment. It is self-evident that transportation does alter the requirement for constant temperature control. Storage at higher temperatures reduces the posttransfusion survival of erythrocytes. Such deterioration is produced by the warming of the blood for a few hours at any time during storage even though it is subsequently refrigerated. The danger inherent in transportation without special equipment for refrigeration is that such a period of warming may occur with resultant damage to the blood. Two factors operate in bringing this about: the temperature of the ambient air, and time. The use of mechanical devices or ice chests is not always necessary. In a cool atmosphere (2° to 10° C.) blood may be moved any distance without special equipment. Likewise it may be moved short distances at any temperature. The universal practice of carrying unprotected blood through hospital corridors or even short distances between hospitals just before transfusion is not contraindicated. Movement involving longer periods will usually require refrigeration.

REACTIONS AND TRANSPORTATION

Since transportation has no obvious effect, one would expect no greater reaction rate from blood which has undergone shipment than is normally encountered. DeGowin and Hardin[5] re-

supply during the Spanish Civil War.[2] Transfusion services became an integral part of the medical organizations of the forces of Russia, the British Empire, France, and the United States during World War II. One of us (RCH) became thoroughly familiar through close association with the operation of these units in the armies of the last three in Europe. The first record of such activity was published in 1940[3] and numerous more complete accounts have since appeared. These operations involved the movement of hundreds of thousands of bottles of whole blood. Shipment by speed boat, landing ship, naval transport, railway, motor vehicle, and aircraft was routine and distance was no barrier. The difficulties encountered were no different, except perhaps in magnitude, than those met in transportation of blood from one hospital to another. The same principles are involved when one bottle of blood is carried a few city blocks as when two tons of blood are flown across an ocean.

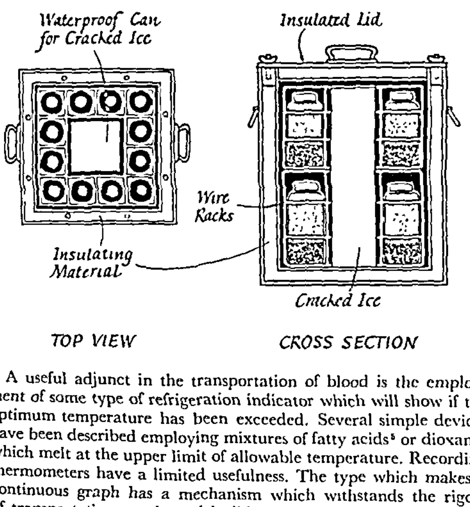
EFFECTS OF TRANSPORTATION

Of primary interest is the determination of whether special conditions encountered in transportation may produce deleterious effects. The two factors which might conceivably alter whole blood are shaking and exposure to low atmospheric pressure.

Effects of Shaking. Shaking of erythrocytes is known to produce rupture. This breakdown is proportional to the amount of shaking but is lessened by the presence of plasma. There is, furthermore, a considerable species difference in the resistance of red blood cells to trauma. The human erythrocyte withstands shaking very well.[4] The solution of the matter lies in determination of whether transportation will produce enough agitation to exceed cellular resistance. DeGowin and Hardin[5] measured the amount of free hemoglobin in the plasma of bloods before and after shipment. Properly refrigerated blood was transported by air and ambulance for distances of 3539 and 720 miles respectively. The blood had been preserved for varying lengths of time (none to eighteen days) in the modified Rous-Turner mixture[6] before shipment. Negligible increases in the free hemoglobin content of plasma were found. It was also noted that blood transported by air underwent sedimentation, which is evidence that shaking was minimal. It was subsequently observed by one of us (RCH) that the agitation encountered in all types of transportation is insufficient to maintain cell suspension. It may be concluded that shaking of blood during transport does not produce enough trauma to erythrocytes to result in hemolysis. The posttransfusion survival of

refrigerant. Excessive amounts will freeze the blood. Amounts incapable of producing freezing do not give adequate refrigeration except for very short periods in small chests but in larger boxes will be found satisfactory. The method is relatively expensive. Mechanical refrigeration has its greatest use in road transport of large quantities of blood for long distances. Refrigerators used for this purpose should be of the type specifically built for mounting on trucks. The household type is not rugged enough to operate successfully for very long under the stress which is encountered. The best type of refrigerator for this purpose is one equipped with a compressor unit, powered by a gasoline engine. The mechanism should be thermostatically controlled.

TYPE OF CHEST USED FOR TRANSPORTING BLOOD



A useful adjunct in the transportation of blood is the employment of some type of refrigeration indicator which will show if the optimum temperature has been exceeded. Several simple devices have been described employing mixtures of fatty acids[5] or dioxane[7] which melt at the upper limit of allowable temperature. Recording thermometers have a limited usefulness. The type which makes a continuous graph has a mechanism which withstands the rigors of transportation poorly and legible records do not always result. The mercury maximum-minimum recording type of thermometer does very well but will not indicate the length of time either extreme

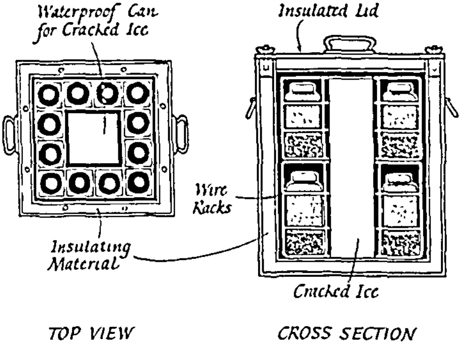
ported forty transfusions of blood transported under constant refrigeration, with chills and fever in one case. Gibson *et al.*,[7] in recording reactions from bloods transported with good refrigeration, noted that in ten transfusions two recipients with allergic histories exhibited anaphylactoid reactions and three had symptoms attributed to rapid breakdown of erythrocytes. Four bloods transported without refrigeration were transfused and, in one case, rapid breakdown of red cells was observed. This high rate of reaction with refrigerated blood is probably coincidental and magnified by the smallness of the series. Hardin[8] collected statistics on 9392 transfusions of blood (universal donor) transported from a central bank to several hospitals, with a reported incidence of reactions of all types of 4.8 per cent. This compares favorably with reaction rates reported from institutions where transportation is not a factor (Chap. 12). It is our opinion that transportation under proper conditions does not affect the incidence of transfusion reactions.

METHODS OF REFRIGERATION

The maintenance of the proper temperature of blood while in transit is really an engineering function and any troublesome problem is worthy of consultation with a refrigeration expert. The equipment used may be divided into four types: (*a*) watertight containers in which the bottles of blood are immersed in ice water, (*b*) insulated chests, (*c*) insulated chests with ice and (*d*) mechanical refrigerators. DeGowin and Hardin[5] utilized the first in their study. The containers employed were ordinary 10-gallon milk cans covered with insulating jackets. The prime requisite for this type of refrigeration is an unquestionably impervious bottle closure. Ice may be replenished as necessary. The refrigeration obtained is entirely adequate. The method is one employing readily available equipment and thus is very useful in an emergency. Insulated chests without ice may be used to a limited extent in short hauls during which the temperature of the blood may be kept adequately low by the simple exclusion of heat. It may be said that the efficiency of this type of chest increases, up to a certain point at least, with size. Larger volumes of chilled blood absorb more heat than one or two bottles with proportionably less individual rise in temperature. The insulated chest with ice is perhaps the most useful method of refrigeration. This may be constructed of any suitable material in a size convenient for manual handling. A watertight compartment or container should be provided for the ice. Solidified carbon dioxide (dry ice) is not the most satisfactory

refrigerant. Excessive amounts will freeze the blood. Amounts incapable of producing freezing do not give adequate refrigeration except for very short periods in small chests but in larger boxes will be found satisfactory. The method is relatively expensive. Mechanical refrigeration has its greatest use in road transport of large quantities of blood for long distances. Refrigerators used for this purpose should be of the type specifically built for mounting on trucks. The household type is not rugged enough to operate successfully for very long under the stress which is encountered. The best type of refrigerator for this purpose is one equipped with a compressor unit, powered by a gasoline engine. The mechanism should be thermostatically controlled.

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of temperature obtained. There is no substitute for frequent inspection by trained personnel.

PROTECTION AGAINST FREEZING

During transportation blood may be exposed to atmospheric temperatures below its freezing point for considerable periods. Since freezing renders blood unfit for administration it must be protected against this eventuality. The insulated chest is as capable of containing heat as it is of excluding it and under relatively mild conditions may give adequate protection. If heat need be used to prevent freezing it should be applied externally to avoid possible overheating if the chest is small in volume. In aircraft the cargo compartment is heated, which obviates this difficulty. In larger mechanical refrigerators cans of hot water or the ordinary kerosene lantern may be placed inside the storage compartment to maintain adequate temperatures. Such a procedure requires constant attendance. One of the authors (RCH) has had the unique experience of being forced to equip large refrigerators with automobile heaters. This combination refrigerator-incubator works very well and can be automatically controlled.

SUMMARY

Transportation *per se* has no effect upon blood. The principles pertaining to ordinary preservation and storage need to be strictly observed in order that the blood may arrive at its destination in a useful state. Conditions met in transport require special consideration and equipment in order that this end may be accomplished.

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CHAPTER 15

Blood Plasma: General Considerations

By John B. Alsever

The indications for the use of blood plasma, its relative merit in each instance, and the types of plasma best suited to each purpose have been presented in Chapter 2. That plasma is of real therapeutic value is well established. However, the indications for its use have now become far more rational than the claims made by some enthusiasts when it was hailed as a "substitute for whole blood transfusion" a few years ago. Plasma obviously cannot be a completely satisfactory "substitute" for whole blood, since it does not carry oxygen, but it is particularly useful in certain conditions in which whole blood is not readily available or erythrocytes are not required. For example, plasma infusions are effectual in the early treatment of severe burns and in the emergency prophylaxis and therapy of traumatic shock while awaiting transportation of the patient to the hospital or the procurement of whole blood.[1,2]

MERITS OF PLASMA

Blood plasma possesses certain distinct advantages. It can be prepared at small expense; it may be stored for long periods of time and transported great distances without risk of deterioration; and it is available for immediate administration (except in the frozen state). Human plasma, properly prepared, can be administered intravenously without blood grouping and crossmatching, in large and repeated doses if necessary, with little danger of serious reaction. However, there are some hazards inherent in the preparation and handling of blood plasma. It is an excellent culture medium so that great care must be taken to avoid con-

tamination. Also, there are certain unstable plasma proteins which have a tendency to flocculate or lose their specificity. Although the use of plasma essentially eliminates the danger of incompatible antibodies, other types of complications may occur. Pyrogens and bacterial contamination are chiefly responsible for the febrile reactions accompanying the administration of plasma. The employment of aseptic technique during the bleeding of the donor and the use of a closed system throughout the entire processing of plasma will reduce the incidence of bacterial contamination to a minimum. Pyrogens can be excluded by scrupulous care in the preparation of the fluids and equipment used in the processing and administration of plasma. The unstable proteins can be preserved, when necessary, by proper methods of preparation and storage.

PLASMA VERSUS SERUM

In the past there has been considerable discussion concerning the relative merit of serum and plasma for transfusion therapy. Both products have been praised and condemned in the literature. Actually, plasma and serum, if properly prepared, have quite comparable clinical value and use.[3,4,5,6,7] The principal drawbacks to the use of serum are a higher reported reaction rate, even in the hands of skilled workers employing both plasma and serum, and the fact that the preparation of serum cannot be carried on as a part of the operation of a blood bank. The high incidence of reactions (up to 25 per cent) is thought to be caused by a substance formed or freed in the clotting of the blood. The reaction rate is sometimes as high as 100 per cent with fresh serum, but it diminishes as the serum ages, and after four to six weeks of storage approximates that observed with plasma. Self, Thalhimer and Scudder,[6] in a careful comparative study of the use of both plasma and serum, liquid and dried, in the treatment of shock summarized the differences between the two products as follows:

Plasma	*Serum*
1. Lower reaction rate	1. Greater protein content (7 gm. as compared with 5 gm. per 100 ml)
2. Greater yield (2 to 4%)	2. No expense or trouble with citrate solution
3. Contains the fibrinogen and prothrombin	3 Can be readily sterilized by filtration
4. Easily prepared as a by-product of a blood bank	4 Remains clear in storage at 5° to 10° C.
	5 Can be dried more easily
	6 Is theoretically safer (no sodium citrate) for massive replacement therapy in patients with low protein and blood calcium

STORAGE OF PLASMA

The decision of whether to prepare and store plasma for use in the liquid, frozen or dried state will depend on (1) the variety of conditions in which it is to be used, (2) the time of storage required, (3) the circumstances under which it is to be stored, transported and administered to patients, and (4) the equipment and technical personnel available in the processing laboratory. The ready availability of large quantities of blood plasma is primarily of value for the emergency replacement of blood volume in the treatment of shock resulting from hemorrhage and burns. It is well recognized that for this most important purpose liquid, frozen, and dried plasma are of equal therapeutic value.

Liquid Plasma. This is by far the simplest and most economical to prepare, as well as to store and administer. With the addition of 50 per cent dextrose solution to produce a final concentration of 5 per cent it will remain relatively stable, except for a gradual loss of the labile fractions, such as complement, prothrombin, and the antibodies, for a period of at least three years[10] when stored at room temperature (Chap. 16).

Frozen Plasma. This product is somewhat more expensive because it must be rapidly brought to the frozen state, it must be stored at temperatures below −20° C. and it must be rapidly thawed at 37° C. before using.[9] Storage in the frozen state does, however, possess certain definite advantages. The possible duration of storage is probably indefinite, as long as a temperature is maintained at which the unstable fractions remain relatively fixed. Freezing, therefore, is a satisfactory method for maintaining plasma in the fresh state. It is a procedure which provides the blood bank with suitable plasma which is available for the treatment of hemorrhagic disorders or for immunotherapy. Facilities for frozen storage (p. 395) should be made available for such purposes in a well-run transfusion service (Chap. 24).

Dried Plasma. The most expensive and difficult type to prepare, the processing of dried plasma requires special costly equipment and more time and qualified personnel than is necessary with liquid or frozen plasma.[9] However, the product remains stable over a wide range of temperature and presumably for as long as the container remains completely sealed. Since rubber stoppers are commonly used to seal the containers, the deterioration of the rubber usually determines the safe period of storage. The present regulation of the National Institute of Health permits the storage and use of dried plasma over a period of five years. Dried plasma must not be reconstituted to the liquid state until just

before administration, and sterile water must be available as a diluent. The container of dried plasma is usually packaged with a flask of sterile 0.1 per cent citric acid solution to restore the pH of the plasma to normal as well as to serve as a solvent. The storage space required is thus doubled in the standard type of package designed to permit reconstitution to the original volume. Such a package, with a suitable administration set included, is most satisfactory and useful when plasma must be stored under varying climatic conditions, transported frequently, and used outside of a hospital. It was for these reasons that dried plasma, so packaged, was chosen for field use by the armed services in World War II (Chap. 18).

Stability of Plasma Proteins. The rate of deterioration depends on whether the plasma is stored in the liquid state at room temperature or in the frozen or dried state.[11] If plasma is brought to the frozen state within seventy-two hours after the blood is collected and either stored frozen at $-20°$ C. (or below) or dried, the protein components will remain as they were at the time of freezing. If liquid plasma is stored at room temperature, significant changes take place in several of the physiologically important proteins: (1) liquid plasma becomes incoagulable within two to six months, and (2) complement activity and prothrombin have essentially disappeared by the tenth day of room temperature storage. There is, however, no change in the albumin, globulin, or fibrinogen (excluding the anti-hemophilic activity) and no increase in the nonprotein nitrogen in plasma prepared with dextrose (p. 369) after six months at room temperature. Storage of liquid plasma at 5 to 10° C. slows the rate of loss of complement and prothrombin but causes the fibrinogen to precipitate (p. 378). The antibodies against infectious diseases in plasma stored at room temperature evidently diminish in potency quite slowly and do not ordinarily show appreciable change until after six months of storage.

CONCENTRATION OF PLASMA

Isotonic Plasma. This is rather arbitrarily defined as plasma to which a sufficient volume of diluent has been added to reduce the protein concentration to not less than an average of 5 to 6 gm. per 100 ml. The dilution is necessary (1) to prevent clotting of the whole blood by the addition of sufficient citrate solution (usually 50 ml. of 4 per cent sodium citrate), and (2) to stabilize the liquid plasma by the addition of 100 ml. of 50 per cent dextrose solution to every 900 ml. of citrated plasma (5 per cent final concentration). Each 100 ml. of isotonic plasma, therefore, represents approxi-

mately 75 ml. of original plasma when dextrose has been added, or approximately 83 ml. without dextrose. Isotonic plasma is probably the form of choice for routine use. In general, it is rational to administer plasma in a form which is as close as practicable to its natural osmotic value and chemical constitution. Exceptions to this statement are special instances where either more or less than the normal fluid volume per gram of protein is indicated (see the following sections, also Chaps. 2 and 18).

Dilute Plasma. When sufficient fluid has been added to reduce the protein concentration to 4 per cent or less, dilute plasma results. It may, generally speaking, be employed with the expectation of clinical results equally as good as those produced by isotonic plasma, prepared and stored under similar conditions. Plasma is usually processed in the dilute form for one of two reasons; (1) this form is the natural by-product of blood diluted with a preservative solution to permit longer than 5 day storage in the blood bank, or (2) it is desired to reduce the agglutinin titer of monovalent plasma (that prepared from a single blood donation). It is the experience of the authors and others (Mahoney,[11] Elliott[8]) that dilute plasma may be even more satisfactory than the isotonic concentration in the treatment of shock, if the patient is dehydrated when therapy is begun. This is based on the observation that when isotonic plasma is employed in severe shock, it is, in fact, sometimes necessary to administer additional fluid in the form of crystalloid solutions to restore normal fluid balance. The practical disadvantage of dilute plasma is that more space is required for storage, and it is not desirable to employ it in conditions in which the fluid intake must be restricted, as in cardiac disease or edematous states, or when a dehydrating effect is desired, as in brain injuries.

Concentrated Plasma. This is defined as plasma from which sufficient fluid has been removed to raise the concentration of protein to 7 gm. per 100 ml. or more. Its use gives excellent clinical results in certain conditions. It has, however, certain limitations and advantages at variance with those of dilute plasma. It is ordinarily prepared for use as follows:

Reconstitution of Dried Plasma. The desired fraction of the original volume, ordinarily ¼, ⅓ or ½, is attained by adding a proper amount of diluent, thereby producing approximately a 20, 15 or 10 per cent solution of protein. The recommended method is to add the desired amount of diluent to the plasma in the container in which it was dried. The practice, advocated by Hill and his coworkers,[14,17] of removing the dried plasma from the original container and placing it in a smaller container is undesirable

because it leaves the clinician no choice but to administer concentrated plasma, and the transfer from one container to another invites contamination. The latter is admittedly not an important consideration unless the plasma is reliquefied and allowed to stand for some time at room temperature before administration.

Concentration of Liquid Plasma. This can be accomplished by repeated freezing and thawing[18] (Chap. 17) or by the evaporation of water from the plasma in a cellophane bag (Hoyt,[20] Thalhimer,[19] and others). This latter method is least desirable because the danger of contamination is greater than with the other procedures.

Although the use of concentrated plasma has been advocated by Hill and his coworkers as the method of choice in the treatment of shock, its general use for this purpose is not recommended since the bulk of the clinical evidence favors the use of isotonic plasma. A review of the favorable results reported with the use of concentrated plasma in shock shows that a large number of the patients received considerable additional fluid as part of the therapy. Concentrated plasma is indicated only for the treatment of shock complicated by a head injury in which dehydration is desirable, and for therapy designed to raise a low blood protein level (Chap. 2). The investigations of careful workers in the field of experimental shock (Levinson,[12,15,16] Mahoney,[13] and others) have shown that dehydration occurs in traumatic shock. Since there is a fluid loss in addition to hemorrhage in most cases of severe shock, notably in burns and crush injuries, the use of isotonic plasma gives considerably better results than the concentrated form. The observed difference between the two was particularly great when dehydration existed. It has been suggested that the drawing of extravascular fluid, and perhaps even intracellular fluid, into the blood stream by the administration of concentrated plasma may well contribute to the poor results. The rapid increase of blood volume produced by the use of concentrated material is particularly dangerous in patients with cardiac disease or pulmonary infections. However, the augmentation of the blood volume by the employment of concentrated plasma may have a definite place in the resuscitation of patients suffering from prolonged shock when maximum speed is essential, *if* dehydration is not also present.

ESSENTIALS IN PLASMA PREPARATION

Regardless of the method which may be employed for the preparation of plasma, there are certain general requirements which apply in all situations:

Protection of the Donor. The donor's physical condition must be such that the withdrawal of the desired amount of blood will not endanger his health.

Protection of the Recipient. The recipient must be guarded against the diseases transmissible by plasma by determining that the donor is free from such diseases.

Collection of Whole Blood. The drawing of blood must be carried out in an adequately equipped bleeding room and by a sterile, closed technique.

Processing of Plasma in a Closed System. A closed system is defined:

> A closed system is a system which permits the transfer of material from one container to another entirely within the system without contamination through exposure to external conditions. It is accomplished either through an exchange of position of the materials within the system or through the introduction of sterile air as the transfer is being made by the application of negative or positive pressure. All air for replacement must first pass through a suitable bacteria-excluding filter, except that when the transfer is being made in a closed 'sterility room' equipped with mechanical or physical air-sterilizing devices of proven quality, and in operation when the transfer is being made, the air for replacement need not be passed through such a filter. (Minimum Requirements, National Institute of Health, 3d revision, January 10, 1946.)

Hemoglobin Concentration of Plasma. The concentration of free hemoglobin in the plasma must be within safe limits for the administration of large amounts of plasma, as in the treatment of severe burns. A maximum content of 50 mg. of hemoglobin per 100 ml. of plasma has been established as a safe limit by recognized authorities in the field (Subcommittee on Blood Substitutes, National Research Council). A satisfactory method for the measurement of the hemoglobin content of plasma is described on page 391.

Cleanliness of Processing Equipment. All equipment which comes in contact with either the blood or plasma must be scrupulously clean and sterile (p. 555). The glassware must be clear and of good quality, the rubber stoppers and tubing must be suitable for use with biologic products, exposed parts must be adequately covered, and all such equipment sterilized in the autoclave.

Sterility. The plasma must be demonstrated by acceptable sterility tests (Chap. 16) to be sterile in the final container.

Labeling. The label must indicate the identity of the donor, or the plasma pool, and the laboratory, date of preparation, amount and kind of diluent and/or preservative, and the date and results of sterility tests (p. 388).

Filtration. This must always be done at the time of administra-

tion. It is customary to insert a filter in the lumen of the tube of the recipient set which is adequate to remove all particles of such size as to be dangerous when introduced intravenously (pp. 393 and 526).

ISOHEMAGGLUTININS IN PLASMA

The absence of incompatible reactions from dilute, isotonic and concentrated plasma, both pooled and unpooled, had been fairly well established by several workers before World War II. The widespread use of plasma in the treatment of war injuries made it imperative to have conclusive evidence of its safety. The available data were supplemented by much additional work. As a result, it can be said that apparently there are rarely any dangerous untoward reactions from the administration of incompatible plasma, although undoubtedly the possibility does exist. A few workers [26,27] have reported reactions from the isohemagglutinins in group O blood administered to recipients of other groups (p. 245). They possibly are due to an abnormal sensitivity of the recipient's red cells. It may be stated, therefore, that blood plasma may safely be given, without regard to blood group or crossmatching, in any amount required therapeutically. Theoretically, pooled plasma is safer, but the evidence indicates that no reaction is likely even from the use of undiluted unpooled plasma, which may by chance have a high titer of incompatible agglutinins. There is, however, no good evidence regarding the safety of concentrated monovalent plasma in this respect. The contention of one group[56] that "the elimination of pooling would necessitate radical changes in the operation of blood banks, one of which would be the requirement that plasma should be typed and crossmatched or treated with group-specific substances" *is contrary to the experience of several workers* (see following paragraph) and is not necessary in the opinion of the authors. However, if concern does exist in this regard, it is suggested that the use of group-specific substances[26] is the simplest and most satisfactory method to employ in a transfusion service.

A few examples of the data supporting the statements relative to the use of pooled and unpooled plasma will serve to illustrate the points which have been made. Lozner and Newhouser[21,22] reported no evidence of harmful effect due to the administration of incompatible agglutinins in a study of 1000 pools of plasma and 100 monovalent controls, some of them relatively high titered. Weinstein[24] reported only one of a total of fifteen reactions observed in a series of 1500 consecutive transfusions of unpooled plasma in which there might be some question that incompatible agglutinins could have been concerned. Thalhimer and Taylor[23] found no complications

of this type in a study of 1354 pools, of which 355 were small (six to ten donors). No pool showed an agglutinin titer of over 1/80 and, curiously enough, none of the small pools ran over 1/40. They also studied several pools of group O plasma, with anti-A titers as high as 1/256 and anti-B as high as 1/128. No reactions were observed during and immediately after the administration of not less than 500 ml. of plasma intravenously to patients of groups A and B. Agglutinates were not found in the recipient's blood. Miller and Tisdall[24] reported no evidence of such reactions in a series of 10,000 administrations of pooled plasma. Their pools were all small (six to seven donors each). Elliott (personal communication) was unable to find any evidence of reaction to incompatible agglutinins during the repeated administration of large amounts (500 to 1000 ml.) of plasma with high titer agglutinins (1/256 or more) incompatible to the recipients. Plasma injections were made daily or on alternate days for eight or ten times. The experience of the Army and the Navy with the use of millions of 250 and 500 ml. packages of plasma failed to produce a single reported incompatible reaction.

On the other side of the picture, Witebsky *et al.*[26] and Morgan and Lumb[27] reported relatively small numbers of cases in which the use of group O blood, and rarely plasma (p. 432), resulted in severe reactions which were attributed to the transfusion of incompatible agglutinins.

TRANSMISSION OF DISEASE BY PLASMA

As with the use of whole blood (p. 293), there are certain diseases which may be transmitted by the transfusion of plasma. Malaria, syphilis, and infectious hepatitis are the principal offenders. Ordinarily, careful selection of the donor will largely obviate the danger of transmitting malaria or syphilis, and the processing of plasma, including the period of storage routinely involved, actually removes any risk in the case of these two infections.

Syphilis. It has been demonstrated that, in the case of *Treponema pallidum*, spontaneous sterilization occurs in both whole blood and plasma within about seventy-two hours after the collection of the blood. There is, therefore, no risk of transmitting syphilis by the transfusion of liquid plasma which is more than five or six days old. There is only a risk in using plasma in the hospital blood bank when the product is relatively fresh. The spirochetes are, of course, destroyed by freezing so that frozen or dried plasma carries no hazard.[28,29,30,58]

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Malaria. There is relatively little danger of the transmission of malaria by the transfusion of plasma. However, this subject was re-

investigated during World War II when some 13 million pints of blood were being collected from millions of donors by the American Red Cross to be processed into plasma. Lozner and Newhouser,[31] in a small but carefully conducted series of experiments, conclusively demonstrated that the danger from malaria transmission was practically nonexistent under the conditions usually encountered in the preparation and use of plasma. Infection of the recipient by plasma heavily contaminated with plasmodia was apparently possible only with liquid plasma which had been stored less than one week. Two weeks of storage of plasma in the liquid state insured complete freedom from danger. The plasmodia do not survive in frozen or dried plasma.

Homologous Serum Jaundice. The causative virus survives indefinitely in liquid, frozen, or dried plasma. Much has been written in the literature concerning the transmission of infectious hepatitis by transfusion of plasma or whole blood.[32-56,59,60] The occurrence of homologous serum jaundice was first reported in 1937 following the use of convalescent serum. Subsequently it was shown to follow transfusions of blood and plasma. The widespread occurrence of the disease after the use of yellow fever vaccine (containing human serum) among our troops in 1942 raised the interest in the transmissibility of this disease to a high pitch. The large numbers of transfusions given those wounded in the war provided an unusual opportunity to study the incidence, epidemiology, clinical course, and sequelae of homologous serum jaundice. It was estimated that between 1 and 2 per cent of the plasma pools used by the armed forces appeared to have been contaminated with the virus. Scheinberg *et al.*[55] recently reported that the mortality rate among those acquiring the disease in the Army was 0.2 per cent. Brightman and Korns[59] reported that follow-up studies on 649 patients who received transfusions of the surplus Army-Navy plasma following its release for civilian use in 1945, showed a subsequent incidence of homologous serum jaundice of 4.5 per cent. In a concurrent study of deaths attributed to acute hepatitis, fifteen of fifty-one cases had had transfusion therapy, twelve received plasma only.

It has been shown that as little as 0.1 ml. of such plasma will transmit the disease and that transmission by the use of syringes and needles not resterilized between injections has been responsible for many cases of infectious hepatitis. The disease which is transmitted by contaminated blood is not easily distinguishable clinically from the epidemic form. However, there are some differences. The virus causing homologous serum jaundice has been shown to be a different one from that causing the epidemic form.[48] The "transfusion" disease has a very long incubation period of sixty to 120

days. This has made study of the disease difficult in ordinary civilian practice, both because of a lack of facilities for follow-up studies on patients receiving transfusions and because of the epidemiologic problem inherent in such a long period of incubation. In addition, homologous serum jaundice is more frequently severe, is characterized by a long convalescence, and is sometimes fatal.

Prevention. It is exceedingly difficult, if not impossible, to prevent transmission of homologous serum jaundice at the present time. There are no means known today to avoid the chance selection of donors carrying the virus. The infectious agent is present in the blood for some weeks before there is any evidence of the disease in prospective donors, either subjective or objective, as well as for some time afterward. Since the virus survives all ordinary methods of processing and storage of plasma and only a minute amount will transmit the disease, a single infected donor will contaminate an entire pool. Oliphant and his coworkers,[42,43] in a study of the disease in the Virgin Islands, found that irradiation with ultraviolet light killed the virus. Progress is being made in developing a practical method for enabling such a procedure to be routinely employed in the processing of plasma[47,49] and it is hoped it will soon be generally available. However, it is likely that the employment of this method may not be economically within the reach of the average hospital blood bank for some time and, therefore, the use of small pools or monovalent plasma will be indicated as a general practice. Loutit,[51] Scheinberg[54] and others have reported studies which indicate that the danger of transmitting infectious hepatitis is greatly lessened if unpooled plasma is employed and care is exercised in the selection of donors.

Selection of Donor. Scheinberg *et al.*[54] suggested that a prospective donor should be rejected if he (1) has ever had jaundice or hepatitis, (2) has had contact with a person with jaundice during the preceding year, (3) has been hospitalized during the year (he may have received the virus from a syringe), (4) has received transfusions of whole blood, plasma, or human serum within one year, (5) shows fever, icterus, an enlarged or tender liver or spleen on physical examination. These precautions seem reasonable except that (*a*) exclusion of donors with a history of jaundice or hepatitis more than six months before would probably be adequate, and (*b*) the detection of slightly enlarged spleens or livers in a blood donor clinic is hardly practical.

It is recommended, therefore, that prospective blood donors be rejected if there is (1) a history of jaundice or hepatitis within six months, (2) a contact with a person with hepatitis within six months, (3) a history of receiving a blood transfusion or injection

of plasma or human serum within six months, (4) a history of hospitalization within six months, (5) the presence of jaundice on physical examination. Fever, of course, has always been a cause for rejection of donors.

Preparation of Plasma. Since the danger of transmission of homologous serum jaundice is enhanced by the pooling of plasma, the wartime trend toward the employment of pools of twenty-five to fifty bleedings should be quickly reversed and plasma should be routinely prepared from single donations or in pools of from two to eight bleedings, unless and until a satisfactory method for the inactivation of the virus[60] or detection of contaminated donors has been perfected.

It is recommended, therefore, that the use of monovalent plasma, or small pools (two to eight bloods), be made routine in every transfusion service. Also, when donors can be followed for two to three months, the suggestion of Scheinberg *et al.*,[55] that plasma be held for such a period and the health of the donor then ascertained, should also be helpful in lowering the incidence of homologous serum jaundice.

Selection of Patients Receiving Plasma. The recommendations of the Committee on Blood and Blood Derivatives of the Advisory Board on Health Services of the American Red Cross[53] regarding the use of the surplus Army and Navy Dried Blood Plasma, which was released for civilian use after the end of the war, discusses briefly the transmission of infectious hepatitis by plasma transfusions and contains a statement which should be made an inviolate rule in the administration of plasma: "Since it (hepatitis) may be a severe and debilitating illness, physicians should only use plasma where definitely indicated."

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type of equipment, although the principles and steps involved are applicable generally.* Since the commercial vacuum-type container is employed in the great majority of hospitals in the collection and storage of whole blood and the preparation of blood plasma, the methods described in this chapter are, with one exception, particularly concerned with this type of equipment. For those who desire to employ reusable apparatus, the preparation of pooled plasma by the centrifuge method is described in detail for one specific type of such equipment.

Commercial Vacuum-Type Containers

There are available several varieties of commercial vacuum-type containers which are entirely satisfactory for the collection and storage of whole blood and the preparation of plasma. Such equipment is commonly employed in blood banks because it is simple to use and usually less expensive on a small scale than the installation and maintenance of reusable equipment. The minimum requirements for such apparatus are: (1) a proper quality of glass for the preparation and storage of a biologic; (2) an adequate control of the identity of the solutions employed; (3) freedom from pyrogens; (4) the sterility of the manufactured unit; (5) a self-contained vacuum sufficient to draw in more than enough fluid to reach the capacity for which the container was designed; (6) a closure which is designed to facilitate the insertion of needles, both for filling and subsequent aspiration or administration of the contents. This closure maintains an airtight seal after puncture with the 15 or 17 gauge needle employed for filling the container, so that a closed system may be maintained; and (7) the bottle should be of a size and shape proper to fit the standard centrifuge cup (p. 521).

Reusable Equipment

A number of hospitals prefer to employ equipment for the collection and storage of whole blood and the separation of plasma which can be cleaned and reused in the laboratory. Several varieties are available which lend themselves to the maintenance of a closed system and meet the other requirements which are essential in this work (p. 353). The employment of such equipment, however, should not be undertaken unless an unusually well-staffed and well-equipped laboratory is available. The cleaning and preparation of the equipment; the manufacture of the necessary solutions; the adequate control of sterility, pyrogenicity, and composition of solutions requires meticulous work by skilled personnel and a considerable investment in equipment and supplies. The details of the prep-

CHAPTER 16

Liquid Plasma

By JOHN B. ALSEVER

The simplest and most economical method of employing blood plasma on a clinical service is in its liquid form (p. 349). The choice of the method to be used in preparing liquid plasma depends chiefly upon which best coordinates with the procedures employed for the collection, preservation, and administration of whole blood. Several procedures and two general types of equipment are described and recommended particularly. *All of the equipment employed must be completely clean, pyrogen-free and sterile. The entire processing, from the collection of the blood to the final bottling of the plasma, must be carried out in a closed system (p. 353). The selection and examination of the donor and the collection of blood must be carried out in accordance with the standards described in Chapter 10.*

Numerous other methods, and variations of the methods to be described, have been suggested for the preparation of liquid plasma. It is the opinion of the authors that they possess no real advantage over the methods presented here and that many of them are less desirable because of one or more disadvantages, such as: the inability to maintain a closed system; the use of sedimenting agents[18] which interfere with subsequent blood typing and may actually be harmful; the use of special equipment[15] which is usually more expensive and may not lend itself as readily to centrifugation or to storage for potential use as whole blood.

CHOICE OF EQUIPMENT

In a working description of any method for the preparation of blood plasma, the details can be particularly pertinent to only one

type of equipment, although the principles and steps involved are applicable generally.[1] Since the commercial vacuum-type container is employed in the great majority of hospitals in the collection and storage of whole blood and the preparation of blood plasma, the methods described in this chapter are, with one exception, particularly concerned with this type of equipment. For those who desire to employ reusable apparatus, the preparation of pooled plasma by the centrifuge method is described in detail for one specific type of such equipment.

Commercial Vacuum-Type Containers

There are available several varieties of commercial vacuum-type containers which are entirely satisfactory for the collection and storage of whole blood and the preparation of plasma. Such equipment is commonly employed in blood banks because it is simple to use and usually less expensive on a small scale than the installation and maintenance of reusable equipment. The minimum requirements for such apparatus are: (1) a proper quality of glass for the preparation and storage of a biologic; (2) an adequate control of the identity of the solutions employed; (3) freedom from pyrogens; (4) the sterility of the manufactured unit; (5) a self-contained vacuum sufficient to draw in more than enough fluid to reach the capacity for which the container was designed; (6) a closure which is designed to facilitate the insertion of needles, both for filling and subsequent aspiration or administration of the contents. This closure maintains an airtight seal after puncture with the 15 or 17 gauge needle employed for filling the container, so that a closed system may be maintained; and (7) the bottle should be of a size and shape proper to fit the standard centrifuge cup (p. 521).

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dextrose solution for stabilization of fibrinogen, as is now the case with the larger pooling bottles (p. 369).

Aspirating Set. The apparatus used for the aspiration of plasma is like a donor set but with a long, blunt needle substituted for the venipuncture needle. As with the donor set, one of the special valve control mechanisms, or a 17 gauge needle and an ordinary screw clamp, may be employed to control and shut off the flow of plasma. The aspirating cannula should (1) have a hose hub; (2) be 13 or 15 gauge size; and (3) be 6 to 9 inches (15 to 22.5 cm.) long for standard containers up to 1000 ml. size and 15 inches (37 cm.) long for the 2000 ml. pooling bottle. Some are furnished with an open square or blunt tip, others with a closed blunt-pointed tip and a side opening a few millimeters above. Although either is satisfactory, the latter is preferable because it punctures the diaphragm of the stopper more easily and avoids direct suction from below during aspiration of the plasma. Direct suction creates currents which are more likely to disturb the red cell layer than those produced by suction from the side. Heavy-walled rubber tubing is used for assembling the set since it must withstand vacuum. The aspirating cannula is shielded for its entire length by thin-walled rubber tubing (Penrose drain or Gooch crucible tubing) which is tied in place over the hub. Cellophane tubing may also be used. The needle should be well glycerinated so that the tubing will slide up and down easily during the aspiration procedure. The lower end of the protective tubing should be attached to a short length of glass tubing which extends beyond the tip of the needle (10 to 20 mm. is sufficient). Such shielding provides protection and permits handling of the cannula as it is used to aspirate plasma from one or more containers. It materially adds to the ease of operation.

Air Filter. The air which is allowed to enter the bottle from which the plasma is to be aspirated must be passed through a bacterial filter. The type commonly employed consists of a piece of glass tubing, about 10 by 50 mm., filled snugly with sterile cotton and connected to a 17 or 18 gauge needle by a short piece of rubber tubing. The cotton must not be too tightly packed or the passage of air will not be free enough to permit satisfactory aspiration.

Glycerinated Syringe. Specimens for culture are withdrawn from vacuum-type containers by the use of a syringe which has been prepared by coating the plunger with USP glycerine, then assembled, fitted with an 18 gauge, 1½ inch (3.25 cm.) needle, wrapped and sterilized. The glycerin forms a satisfactory seal for the withdrawal of plasma from the evacuated container. A syringe of 10 ml. capacity is sufficient for the 300 ml. plasma unit; and a 50 ml. size is required for the 2000 ml. pool.

aration of plasma by the centrifuge method with one type of available reusable equipment are given on page 378. The principles may be readily applied to the other methods described.

CENTRIFUGE METHOD WITH VACUUM BOTTLES

EQUIPMENT

Centrifuge. The best available centrifuge for the preparation of plasma is a model which has been specifically designed for the purpose, since other laboratory centrifuges of adequate size (International size #1 and #2) are not constructed to carry such a heavy load. Such centrifuges are now standard items of manufacture.

Torsion Balance. A good torsion balance is essential for satisfactory balancing of loaded centrifuge cups. A capacity of 4.5 kg. is adequate.

Refrigeration. It is not essential to employ refrigeration in the preparation or storage of plasma. However, in some acceptable methods, the whole blood is stored at 5° to 10° C. before aspiration of the plasma. The specifications of the refrigerator are the same as those for the preservation of whole blood.

Containers. The essential requirements have been previously described (pp. 353 and 363).

Bleeding Bottle. The 600 ml. size, containing citrate solution with or without a small volume of dextrose preservative, is most often employed for the preparation of plasma. Other sizes may be preferred, depending on the volume of blood to be withdrawn from the donor and on the type of preservative solution employed (pp. 326 and 521).

Pooling Bottle. The 2000 ml. size, containing 200 ml. of 50 per cent dextrose, is most commonly employed when pooling is done with commercial vacuum-type equipment. A 600 and 1000 ml. size can also be obtained, if desired. Containers for pooling can be had empty if it is not necessary to add dextrose in the pooling operation. The need for a pooling bottle depends upon the method of preparation. The size of the pooling bottle is determined by the number of blood collections per pool.

Final Container. An empty bottle, of 300 or 600 ml. capacity, has been ordinarily employed as the final container. The trend has been toward the use of the larger size, since most patients requiring plasma usually need more than 300 ml. Empty containers of this size are standard items on the market. The present emphasis on the use of unpooled plasma (or very small pools) will doubtless encourage the marketing of these sizes with sufficient 50 per cent

dextrose solution for stabilization of fibrinogen, as is now the case with the larger pooling bottles (p. 369).

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Storage of Blood Before Centrifugation

There are three satisfactory procedures which may be followed in the handling of the containers of blood before centrifugation.

Immediate Centrifugation. Satisfactory plasma can be prepared by centrifuging at once, that is within a few minutes to a few hours after collection. If this period is more than a few minutes, the container should be refrigerated if the red cells are to be saved for transfusion. The yield of plasma is somewhat less satisfactory than with a longer period of storage.

Centrifugation After Storage at Room Temperature. Maximum yield is obtained after a twelve to twenty-four hour period of storage. The clarity of the plasma is best when this storage is at room temperature. This is the method of choice if the preparation of liquid plasma is the *sole purpose* for which the blood was collected. *The red cells are not suitable for transfusion.*

Centrifugation After Refrigeration. A period of twelve to twenty-four hours of storage at 5° to 10° C. is the method of choice when it is desired to preserve the unstable protein fractions, maintain the blood in a suitable condition for possible use as whole blood, or use the red cells subsequently in transfusion. The blood should be refrigerated at once for maximum preservation of the red cells and unstable proteins.

There are three important precautions in the handling of blood which is to be processed into plasma: (1) collections of whole blood should never be pooled, as this may produce an undue amount of red cell destruction; (2) freezing of the blood must be avoided as this produces complete erythrocyte disintegration; and (3) bacteriostatic agents should not be added to whole blood since such a procedure is entirely without value. Sterility can easily be maintained with the use of a closed system. In fact, the introduction of bacteriostatic agents into the bottle containing the blood may actually result in contamination.

Centrifugation

Balancing the Cups. The trunnion cups containing the bottles of blood *must* be perfectly balanced before starting the centrifuge. Much blood has been wasted due to breakage, expensive centrifuges have often been damaged, and very unsatisfactory separation of plasma has resulted from the excessive vibration caused by lack of attention in achieving a perfect balance. The weight of 500 ml. of blood is so great that even a slight imbalance will produce sufficient vibration, as the centrifuge is allowed to stop after its run, to cause the red cells to swirl up into the plasma, and the separation will not be satisfactory. *After placing the containers of blood in the trunnion*

cups water should be added up to the shoulder of the bottle before balancing. This reduces the danger of breakage. A good torsion balance should always be employed.

Balancing may be done by either of two methods:

Weighing Loaded Cups Against Each Other. For this method the torsion balance must be absolutely level and, after the initial balance has been achieved, the cups should be exchanged on the balance pans to provide the necessary check of an accurate balance. If the cups fail to balance when exchanged, the scale is not level and must be adjusted. When this procedure has been satisfactorily completed, the two balanced cups should be placed directly opposite one another in the centrifuge. Successive pairs of bottles in cups are then balanced and placed opposite one another until the centrifuge has been filled. An empty blood container should be filled to the proper volume with copper sulfate or other solution of the approximate average specific gravity of whole blood (about 1.058) for balancing, when an odd number of blood flasks are to be centrifuged.

Balancing With a Counterweight. An alternate method of balancing is to place on one balance pan a weight which is slightly heavier than the heaviest trunnion cup and full blood container. Then the heaviest cup and bottle are placed on the opposite pan and balance achieved by adjusting the slide beam weight. The other cups are then balanced to weigh the same as the first. Cups balanced by this method may be paired at random and an exact leveling of the balance is not required.

Achieving Balance. With the first method, this should be done by adding rubber bands of various sizes (or fruit jar gaskets) to the pan holding the lighter cup and bottle. With the second method, the rubbers are added to the pan holding the cup and bottle as each successive one is balanced to the exact weight of the heaviest. After exact balance has been achieved, the rubber bands which have been added to the pan are placed around the neck of the bottle to put the additional weight in the correct location. This provides a similar distribution of weight for each bottle, and is as essential as exact balancing for successful high speed centrifugation.

The Centrifuge Run. When the centrifuge has been properly loaded, it should be started slowly and gradually brought up to the desired speed. Should excessive vibration develop, other than the transient vibration always observed at the critical speed of 500 to 750 revolutions per minute, the cups are not in true balance. The machine should be stopped at once and the cups re-balanced. The blood should be centrifuged for one hour at 2000 to 2500 revolutions per minute. At the end of this period, the centrifuge rheostat is turned down gradually over a period of a few minutes until the

hemolysis [p. 386]), or (*b*) after up to ten days of storage when the blood is collected in one of the small volume dextrose preservative solutions (p. 334).

The Addition of Dextrose. In the preparation of liquid plasma for storage at room temperature, a sufficient volume of fifty per cent dextrose solution must be added to the final container, preferably before aspiration of the plasma, to result in a five per cent final concentration. Therefore 10 ml. are required for each 100 ml. of final volume of plasma; 30 ml. in the 300 ml. bottle and 60 ml. in the 600 ml. (double unit) bottle. If one of the small-volume dextrose preservative solutions is used instead of simple citrate solution, some additional dextrose must be added to achieve the desired final concentration, since these solutions contain only enough dextrose to result in about a 0.3 to 1 per cent concentration. If dextrose solution is to be added to the final container, the addition should be carried out when the plasma is aspirated into the bottle and performed as the first step.

If dextrose is not added to plasma to be stored at room temperature, there will be a gradual precipitation of fibrin which will interfere with, and at times will make almost impossible, the administration of the plasma through the filters in current use.

The Addition of a Bacteriostatic Agent. This is not recommended (p. 387).

Procedure. After completion of centrifugation and the preparatory period of storage, the plasma is aspirated from the bottles of separated blood as follows:

1. Remove the cover of the stopper aseptically. *Handle the blood bottles with care to avoid agitation of the red cells.*

2. Clean the stopper with tincture of iodine and alcohol, with particular attention to the areas through which needles will be inserted for aspiration. These are usually indicated by a depressed or raised area which requires more careful cleaning. Keep the stopper covered with an alcohol sponge to preserve sterility until the operator is ready to carry out the succeeding steps.

3. Clean the closure of an empty vacuum container of the proper size in the same manner.

4. Release the vacuum in the blood container by the insertion of a suitable sterile air filter (p. 365) in the position indicated by the manufacturer. The air filter is left in position to provide filtered air for replacement as the plasma is withdrawn. The alcohol sponge should still cover the area to be used for insertion of the aspirating cannula.

5. Open a sterile aspirating set (p. 365) and insert the aspirating cannula, shielded by thin tubing, into the blood bottle, in the

speed reaches about 800 revolutions per minute. At this point the power should be cut off and the brushes disengaged to permit a slow, unbraked, smooth stop. A rapid stop, even with quite gentle braking when the speed is almost zero, will cause the red cells to swirl up into the plasma and the separation will be unsatisfactory. Occasionally there will be slight swirling even with the most careful technique. The bottles must be removed with care for the same reason.

Storage Prior to Aspiration. For maximum settling of the cells and clarity of the plasma, especially if any visible swirling has occurred, it is advisable to allow the bottles to stand for six to twelve hours at the storage temperature employed prior to centrifugation before proceeding with aspiration of the plasma.

The Aspiration of Plasma

The Closed or Sterile Room. Although the use of a closed or sterile room for the aspiration and transfer of plasma by the methods described in this chapter has not been found to be essential (particularly when the commercial vacuum-type equipment is employed), the use of a closed dust-free cubicle is certainly an advantage. It undoubtedly reduces the possibility of contamination and aids in maintaining a proper respect for the cleanliness and sterile technique essential to the handling of any biologic. The use of a closed sterile room, in which only filtered air is circulated and where sterilization of the air, the work area and the apparatus is accomplished by ultraviolet light, is desirable when reusable equipment is employed, and is essential when a closed system cannot be maintained, as is the case in the preparation of dried plasma (p. 409).

Size of the Final Container. The size of the final container employed for the storage and use of plasma is largely a matter of choice for the blood bank. The common sizes are: 100 to 150 ml. (usually for pediatric use); 250 to 300 ml. (the so-called *single unit*, containing the volume of plasma obtained from one 500 ml. blood donation); 500 to 600 ml. (the *double unit*, preferred in most banks for routine use, since most patients require at least this volume); 1000 ml. (this may be employed to contain a *double unit* of dilute plasma).

Aspiration Directly into the Final Container. The preparation of unpooled or *monovalent* plasma by centrifugation is likely to be carried out most often in the blood bank to recover plasma from outdated whole blood: (*a*) within a maximum storage period of three days when the blood is collected in citrate solution (centrifugation after a longer period will usually result in excessive

plasma which is prepared from freshly drawn blood and administered at once. The specimen for culture may be taken from the final container after transfer of the plasma or from the blood bottle, using the plasma remaining in the aspirating set at the time of transfer. The former method is to be preferred for it proves the sterility of the plasma *in* the final container. The culture medium usually employed is Brewer's sodium thioglycollate medium (p. 392) and it may be prepared for use in either cotton-stoppered or sealed evacuated test tubes or bottles. *An accurate permanent record of all cultures should be kept.*

Use of Cotton-Stoppered Culture Tubes or Bottles. This requires careful stoppering of the tube or bottle before sterilization, dust-free storage and extreme care in opening for inoculation and restoppering to avoid accidental contamination.

Use of Vacuum-Type Culture Tubes or Bottles. This method entails the preparation of culture tubes or bottles which are closed by a perforable rubber stopper with a vacuum drawn before sterilization. The technique, described by Strumia,[4] is quite satisfactory since it practically eliminates false positive cultures from accidental contamination. The stoppers are sterilized for inoculation by cleaning with iodine and alcohol and covering with an alcohol sponge until ready to use, as has been described.

Culture of the Final Container. This is the preferred method. It is recommended that the material for culture should not be taken from the final container until it has been allowed to stand at room temperature for twenty-four to forty-eight hours. This procedure permits any chance contaminant to grow sufficiently to obviate *almost completely the danger of obtaining a falsely negative culture,* which is possible at the time of aspiration. Although false negative cultures are seldom encountered in well-run blood banks, they do occasionally occur, and the potentially disastrous effects of releasing contaminated plasma for use makes the precaution well worth while. False negative cultures have been eliminated in laboratories which have adopted this routine. To take the culture specimen, proceed as follows:

1. Remove the cover of the stopper of the final container, carefully clean it with iodine and alcohol, and keep it covered with an alcohol sponge, as previously described (step 2, p. 369).

2. Unwrap a previously prepared, sterile, glycerinated syringe (p. 365) (for the unpooled plasma unit, one with a capacity of 10 ml.) to which a sterile 1½ inch (3.25 cm.) 18 gauge needle has been attached. *Be sure the fit is tight and leak-proof.*

3. Insert the needle through the self-sealing portion of the stopper previously employed in filling the container.

position indicated by the manufacturer, so that the tip is about 3 cm. *below the surface of the plasma.*

6. Remove the protecting cover from the short, large bore needle (usually 15 or 17 gauge) on the opposite end of the aspirating set and insert it into the final container, in the position indicated by the manufacturer, *with the shut-off valve or clamp closed.*

7. Begin the aspiration by opening the valve or clamp. At first, a rapid rate of flow may be permitted but, when the cannula has been lowered to about 3 cm. from the red cell layer, the flow must be slowed considerably to avoid disturbing and aspirating cells. The end of the cannula must always be kept below the plasma surface while the aspiration is proceeding or the vacuum will be dissipated and the container cannot be filled.

8. Insure good illumination for the aspiration procedure. A strong beam of artificial light should be focused at the junction of the red cell and plasma layers to assure prompt detection of a disturbance of cells before they are drawn into the cannula.

9. Close the valve or clamp and raise the tip of the cannula above the plasma surface when no more plasma can be removed without stirring up the cells. This ordinarily leaves a layer of plasma 0.5 to 1 cm. in thickness.

10. If delays occur in carrying out the steps outlined above, the cleaned tops of the containers should be protected by covering them with alcohol sponges.

11. The aspirating set may be employed for the transfer of plasma from additional bottles of blood as follows: the flask of blood and empty container should be prepared in advance as described in steps 1, 2, 3, and 4; then with the valve or clamp closed, the short needle is transferred to the next empty plasma bottle; the aspirating cannula is then transferred to the next blood bottle, and the aspiration carried out as described. This method has been found satisfactory for handling up to ten bottles of blood without contamination.

12. Invert the filled containers repeatedly to mix thoroughly the plasma with the dextrose solution.

13. After all final containers have been filled and mixed, clean the tops of the stoppers with an alcohol sponge and replace the cover of the stopper. If this can not be done satisfactorily, a suitable covering should be applied to protect the stopper and keep it clean during storage.

Test for Sterility

Each unit of plasma must be demonstrated to be sterile before it is released for storage and future use. It is not necessary to culture

plasma which is prepared from freshly drawn blood and administered at once. The specimen for culture may be taken from the final container after transfer of the plasma or from the blood bottle, using the plasma remaining in the aspirating set at the time of transfer. The former method is to be preferred for it proves the sterility of the plasma *in* the final container. The culture medium usually employed is Brewer's sodium thioglycollate medium (p. 392) and it may be prepared for use in either cotton-stoppered or sealed evacuated test tubes or bottles. *An accurate permanent record of all cultures should be kept.*

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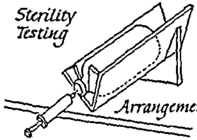
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3. Insert the needle through the self-sealing portion of the stopper previously employed in filling the container.

4. Invert the bottle and aspirate 4 to 8 ml. of the plasma. *Any leakage during this step will permit the entry of unfiltered air and, except in a sterile room, may result in contamination of the plasma.* A simple support, which holds the inverted bottles at a 30 to 40 degree angle is most useful (see figure below).



5. Use two culture tubes or bottles containing Brewer's sodium thioglycollate medium (p. 392) and inoculate 2 to 4 ml. of plasma into each from the glycerinated syringe, removing the cotton stopper or piercing the rubber diaphragm as the case may be. Each tube (approximately 25 by 150 mm.) or bottle (50 ml.) contains 20 ml. of medium. This is sufficient for the 2 to 4 ml. inoculum and contains enough thioglycollate to inactivate the bacteriostatic agents, if they have been added as described on page 387.

6. Place one tube at room temperature (20° to 25° C.) and the other in an incubator at 37° C.

7. Carry out this procedure with each final container.

8. Obtain sterile specimens for other desired tests in the same manner as described for the culture specimens.

Culture of the Blood Bottle. Although this technique is acceptable and is simpler to carry out, it is not so desirable as the preceding method, for it only demonstrates the sterility of the plasma as it comes from the blood bottle. The final container could still be contaminated.

1. Prepare two culture tubes or bottles for inoculation as soon as the aspiration of the plasma from the blood bottle has been completed and *before* transfer of the aspirating set to the next blood bottle and final container (step 5, this page).

2. Inoculate 2 to 4 ml. of plasma into each tube or bottle by withdrawing the short needle from the final container, releasing the valve or clamp and closing it again when the desired amount of plasma has been delivered into the culture tube or bottle (step 5, this page).

3. Transfer the aspirating set, as previously described in step 11, page 370.

4. Place one tube at room temperature (20° to 25° C.) and the other in an incubator at 37° C.

5. Carry out the procedure on each bottle of blood after aspiration of the plasma.

Release of the Plasma for Use. 1. Observe the cultures for ten days. The plasma may then be released for use if the cultures are negative.

2. If either or both cultures contain bacterial growth, the culturing must be repeated from the final container with two additional tubes handled as described. If either of the repeat cultures shows growth, the plasma must be discarded.

Pooling of Plasma

Although the pooling of plasma has been usual and is most economical in time and equipment in performing sterility tests and other control procedures, it multiplies the danger of the transmission of infectious hepatitis with the present methods (p. 358).

The pooling of centrifuged plasma is commonly carried out when the whole blood has been collected and stored in citrate solution or one of the small volume dextrose preservatives (p. 334). Pooling is not ordinarily attempted when large volume preservatives are employed in the storage of whole blood in the bank.

Size of the Pool. Pools may vary in size from the plasma from two bleedings, about 500 ml., to that from 50 bleedings, the size employed in the American Red Cross program during the war. With the use of commercial vacuum-type containers, a 2000 ml. bottle is the largest standard size. With reusable equipment, larger bottles are available which will permit the pooling of ten to twenty-five or more bloods. Because of the danger of homologous serum jaundice, the use of large pools is definitely contraindicated until control measures become available. Thus the size of the pool to be prepared depends on an evaluation of the factors previously discussed in Chapter 15, and on the type of equipment to be used.

Addition of Dextrose to the Pool. In the preparation of pooled liquid plasma from citrated blood for storage at room temperature, a sufficient volume of 50 per cent dextrose solution must be added to the plasma to result in a 5 per cent final concentration. Therefore, 100 ml. is required for each 1000 ml. of final volume of pooled plasma. The 2000 ml. pooling bottle offered on the market as a vacuum-type container can be obtained with or without the required volume (200 ml.) of 50 per cent dextrose solution. Doubtless, smaller sizes can and will be made available with dextrose for those desiring smaller pools. If the blood has been collected

in one of the small volume dextrose preservative solutions, additional dextrose must be added to achieve the desired final concentration, since these solutions contain only enough dextrose to make a 0.3 to 1 per cent concentration. If dextrose solution is to be added to the pooling bottle, the addition should be carried out in the same manner as when the plasma is aspirated into the bottle, and it should be performed as the first step.

If dextrose is not added to plasma to be stored at room temperature, there will be a gradual precipitation of fibrin which will interfere with, and occasionally make almost impossible, the administration of the plasma through the standard filter sets in current use.

Procedure. After centrifugation and the preparatory period of storage, the selected number of bottles of centrifuged blood are processed as follows:

1. Remove the cover of the stopper of each bottle aseptically. *Handle the blood bottles with care to avoid agitation of the red cells.*
2. Clean the stopper with tincture of iodine and alcohol, with particular attention to the areas through which needles will be inserted for aspiration. These are usually indicated by a depressed or raised area which requires more careful cleaning. Then keep the stopper covered with an alcohol sponge to preserve sterility until the operator is ready to carry out the succeeding steps.
3. Prepare the top of the pooling bottle for use in a similar manner.
4. Release the vacuum of the first bottle of blood by the insertion of a suitable sterile air filter (p. 365) in the position indicated by the manufacturer. The air filter is left in place to provide filtered air for replacement as the plasma is withdrawn. The alcohol sponge should still cover the area for insertion of the aspirating cannula.
5. Open a sterile aspirating set (p. 365) and insert the aspirating cannula into the blood bottle in the position indicated by the manufacturer so that the tip is about 3 cm. below the surface of the plasma.
6. Remove the protecting cover from the short, large bore needle (usually 15 or 17 gauge) on the opposite end of the aspirating set and insert it into the pooling bottle, in the position indicated by the manufacturer, *with the shut-off valve or clamp closed.*
7. Begin the aspiration by opening the valve or clamp. At first, the permitted flow may be quite rapid but, when the cannula has been lowered to about 3 cm. from the red cell layer, the rate of aspiration must be slowed considerably to avoid disturbing and aspirating cells. The end of the cannula must always be kept

below the plasma surface while the aspiration is proceeding or the vacuum in the pooling bottle will be dissipated and prove ineffectual.

8. By proper illumination assure prompt detection of disturbed cells before they are drawn into the cannula. A strong beam of artificial light should be focused at the junction of the red cell and plasma layers.

9. When no more plasma can be removed without stirring up the cells, ordinarily leaving a plasma layer of 0.5 to 1 cm., close the valve or clamp and raise the tip of the cannula above the plasma surface.

10. If delays occur in carrying out the steps outlined above, protect the cleaned tops of the containers by covering them with an alcohol sponge.

11. Release the vacuum in the next bottle of blood as described in step 4. Then, with the valve or clamp closed, transfer the aspirating cannula and aspirate in like manner. The procedure is repeated until the desired number of bottles have been added to the pool.

12. Invert the pooling bottle repeatedly to mix thoroughly the plasma with the dextrose solution.

13. Clean the stopper of the pooling bottle with iodine and alcohol and replace the cover of the stopper. If this cannot be done satisfactorily, a suitable cover should be applied to protect the stopper and keep it clean during storage.

The Addition of a Bacteriostatic Agent. This is not recommended (p. 387).

Sterility Test on the Pool. It is recommended that the specimen for culture should not be taken from the pool until it has been allowed to stand at room temperature for twenty-four to forty-eight hours. This procedure permits any chance contaminant to grow sufficiently to obviate almost completely the danger of obtaining a falsely negative culture (p. 371). *An accurate and permanent record of all cultures should be kept.*

1. Remove the cover of the stopper of the pooling bottle, carefully clean the surface of the closure with iodine and alcohol and keep it covered with an alcohol sponge, as previously described (step 2, p. 374).

2. Unwrap the previously prepared, sterile, glycerinated syringe (p. 365) (50 ml. capacity for a 2000 ml. pool) to which a sterile 1½ inch (3.25 cm.) needle has been attached. *Be sure the fit is tight and leak-proof.*

3. Insert the needle through the self-sealing portion of the pooling bottle stopper previously employed in filling the container.

4. Invert the pooling bottle and aspirate 20 ml. per 1000 ml. of the pool (40 ml. in the customary 2000 ml. pool). Additional plasma, which may be desired for other tests, may be withdrawn in the same manner with another glycerinated syringe. *Any leakage during this step will permit the entry of unfiltered air and, except in a sterile room, may result in contamination of the pool.* A simple support which will hold the inverted pool bottle at a 30 to 40 degree angle is most useful (see figure on p. 372).

5. Use four tubes, or bottles (p. 372), of 100 ml. capacity and about 6 by 17 cm. in size, each containing 50 ml. of sodium thioglycollate medium (p. 392). This is sufficient medium for the 10 ml. inoculum and contains enough thioglycollate to inactivate the bacteriostatic agents if they have been added as described on page 387. Inoculate 10 ml. of plasma into each one from the glycerinated syringe.

6. Place two culture tubes in an incubator at 37° C., keep the other two at room temperature (20° to 25° C.) and observe over a period of ten days.

7. If one or more tubes show growth, the culture procedure must be repeated with at least one final container selected at random (p. 377).

FILLING FINAL CONTAINERS FROM THE POOL

The plasma pool may be distributed into final containers of the desired size at any time after the pool culture has been taken. It is customary to distribute the pool immediately after culturing.

Procedure.

1. Re-clean the top of the stopper of the pooling bottle with iodine and alcohol sponge as previously described.

2. Expose the stoppers of the final containers, clean, and cover in the same manner.

3. Release the vacuum in the pooling bottle by the insertion of a suitable air filter in the position indicated by the manufacturer. Leave the filter in place to provide filtered air for replacement as the plasma is withdrawn. The alcohol sponge should still cover the area for insertion of the aspirating cannula.

4. Open the sterile aspirating set (p. 365) and insert the aspirating cannula, shielded by thin tubing, in the position indicated by the manufacturer, so that the tip is at least 3 cm. below the plasma surface.

5. Place the large bore needle, *with the valve or clamp closed*, through the stopper of the final container in the position indicated by the manufacturer, open the valve or clamp, and close it when the final container is filled to the desired volume.

6. Repeat the filling procedure with each final container. Approximately 5 ml. for every 100 ml. of original pooled plasma is withdrawn into a final container designated as the *pilot bottle*. One half the contents is drawn from the pool at the beginning of the filling of final containers and the other half at the end of filling. No more than 150 ml. is required altogether.

7. After the pool has been distributed and the pilot bottle filled, clean the tops of the stoppers of the final containers with an alcohol sponge and replace the stopper covers. If this cannot be done satisfactorily, a suitable covering should be applied to protect the stoppers and keep them clean during storage.

8. Label suitably all final containers, including the pilot bottle (p. 388), and store at room temperature, preferably with a minimum of exposure to direct sunlight (p. 378).

The Pilot Bottle. In filling final containers from a pool it is essential to prepare and retain a sample of the pool in the pilot bottle for sterility testing of the final containers before releasing them for use. In preparing the pilot bottle it is recommended that about one half of the plasma it contains should be taken first from the top of the pool and the remainder from the bottom of the pool. Therefore, sufficient plasma should be left in the pooling bottle after filling the final containers. The amount of plasma for the pilot bottle depends to some extent on the size of the pool. Fifty milliliters for every 1000 ml. of pooled plasma is a sufficient volume but no more than 150 ml. is necessary in large pools.

The pilot bottles should be retained after the sterility testing (described in the following paragraph) as a control for each pool of plasma so that additional studies may be done if indicated by reactions experienced in using the plasma. It is recommended that each pilot bottle be kept until all the plasma in the particular pool has been used. As a number of pools are used and the pilot bottles accumulate, they may be pooled, cultured, and issued for use if so desired.

Sterility Test on the Final Containers. This test is run on the plasma in the pilot bottle after it has stood at room temperature for at least twenty-four hours. The culture of this plasma is carried out in the manner described for "Sterility Test on the Pool," page 375. *If the cultures of the pilot bottle or the pool show bacterial contamination, all final containers of this lot of plasma should be carefully examined and at least one must be subjected to sterility testing unless contamination is evident by inspection.*

Release of the Pool for Use. The final containers filled from a pool of plasma may be released for use if the cultures of the pool and pilot bottle show no bacterial growth in ten days. If contami-

nation of the pool or the pilot bottle is confirmed by culture of one or more of the final containers, the pool must be discarded (except for such individual containers that may be proven to be sterile by culture).

STORAGE OF LIQUID PLASMA

The storage of liquid plasma should be carried out at room temperature. The preferable range is 15.5° to 26.6° C. (60° to 80° F.); the maximum limits are 13° to 37.8° C. (55° to 100° F.). It is recommended that there be a minimum of exposure to direct sunlight, since this may raise the temperature of the plasma above the desired range. With these precautions liquid plasma can be kept in a condition suitable for clinical use for at least three years.

The evidence for this storage period has been largely derived from studies conducted by or under the auspices of the U. S. Navy during its wartime plasma program.[13,14] A study of 3384 reports on the use of plasma, stored at room temperature for varying periods beyond one year, showed it to be safe and quite effective in replacement of blood volume after at least three years of storage. This plasma was prepared at the National Naval Medical Center by the centrifuge method, employing the commercial vacuum-type containers essentially as described in this chapter. It is interesting that the reaction rate with this plasma was significantly less than that with a comparable series of 1500 administrations of commercially prepared dried plasma. Presumably this may be considered as evidence that it is more difficult to prevent pyrogen contamination with reusable equipment. The chemical and physicochemical changes at the end of three years of storage were of no clinical significance. The detailed studies showed no gross change in the total protein content of the plasma. The nonprotein nitrogen increased slightly. The albumin and globulin remained essentially unchanged quantitatively (98 to 99.5 per cent), although electrophoretic and osmometric studies demonstrated a limited amount of protein cleavage.

The storage of liquid plasma under refrigeration at 4° to 12° C., the usual procedure for biologics, will result in the precipitation of fibrin which will interfere with or, at times, make nearly impossible the administration of such plasma through the standard filters.

CENTRIFUGE METHOD WITH REUSABLE EQUIPMENT

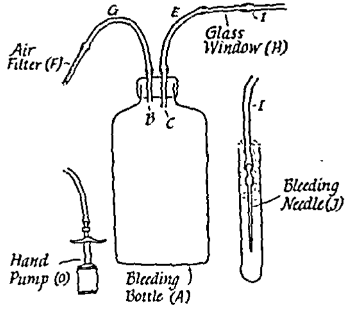
The technique and apparatus to be described in the following sections is that developed by Strumia.[5,6] It provides for the maintenance of a closed system throughout the procedure and has been

found to be a satisfactory method. Other varieties of reusable equipment can be employed with minor variations. In order to present in detail the technique of maintaining a closed system throughout the process, a description of the apparatus and its use is given here, including the collection of blood. For general requirements see pages 353 and 363.

COLLECTION AND PREPARATION OF WHOLE BLOOD

Collecting Equipment. The following parts are required for the assembly of the apparatus (see figure below) for a single collection of blood:

Apparatus for Blood Collection



1. A bottle made of high grade hard glass, 9.2 cm. in diameter (3⅝ inches), 16.6 cm. in height (6⅝ inches), with a capacity of approximately 650 ml. This bottle will fit standard centrifuge cups, and is so built as to withstand high speed centrifugation (2500 revolutions per minute). The neck is short and has an inside diameter of 26 mm.

2. A hooded, two hole rubber stopper fitting the bottle just described.

3. Two pieces of glass or stainless steel tubing *B* and *C*, 7 mm. outside diameter, and 3 and 4 cm. long respectively.

4. Two pieces of transparent amber rubber tubing, 8 cm. long with an outside diameter of 4.8 mm. and a wall thickness of 1.6 mm., *G* and *E*, connected to tubes *B* and *C*.

5. One air filter *F* consisting of a glass tube about 10 mm. by 50 mm., with both ends slightly constricted by flaming. This tube is filled with cotton, and connected with rubber tube *G*.

6. One glass window *H*, consisting of a glass tube about 50 mm. long and 6 mm. outside diameter, connected with rubber tube *E*.

7. One piece of rubber tubing *I* of the same size mentioned under *4*, 60 cm. long, connected to glass window *H*.

8. One bleeding needle *J*, 15 gauge, with a round hub to fit rubber tube *I*. The needle is protected by a 15 by 100 mm. glass tube firmly fitted with cotton around the hub of the needle *J*.

9. A plain rubber stopper to fit a 15 by 100 mm. test tube.

10. A cloth pocket, with tapes for tying, to hold a 15 by 100 mm. test tube. After proper cleaning of the glass and rubber parts, the desired solution is introduced in the bottle. The set is then assembled as shown in the figure on page 379. Care is taken so that tubes *B* and *C* project exactly 12 mm. above the hooded rubber stopper. Rubber tubes *G* and *E*, when fitted to tubes *B* and *C*, must cover their entire exposed portion and be in contact with the hooded rubber stopper. This permits some tendency to adherence to the stopper after sterilization and prevents slipping off later.

The assembled set is placed in a bag of unbleached muslin with the small rubber stopper. The bag is closed, and the apparatus steam-sterilized.

If the set is to be transported outside of the institution, it is desirable to close the rubber tubes *G* and *E* after sterilization. This is accomplished by clamping them through the bag with a stout pinch clamp.

Collection, Storage and Centrifugation of the Blood. During the collection of blood, the bottle is maintained as far below the height of the patient's arm as permitted by the length of rubber tube *I*. Generally, the blood flows well by gravity, but initially the flow may be started by suction with a small hand pump *O* applied to air filter *F*. When a free flow of blood is established the hand pump may be removed. During the collection of blood the bottle should be agitated by a gentle rotating motion, to insure thorough mixing with the anticoagulant solution (Chap. 10).

When the full amount of blood has been collected, release the tourniquet, pinch tube *E*, and remove the needle from the vein. Place the needle inside the test tube, and by gentle milking, discharge 5 to 6 ml. of blood from the rubber tube. The test tube is immediately stoppered, and stored in the cloth pocket. This is tied to the neck of the bottle along with an identification tag. Next, the rubber tubes *G* and *E* are folded down with a slight pull and fixed tightly against the neck of the bottle with two stout rubber bands

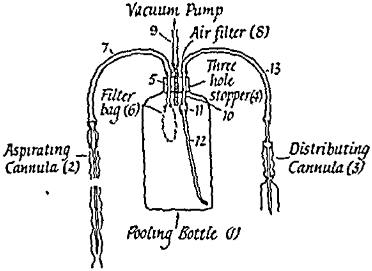
to form a seal. However, excessive stretching of the rubber tubes should be avoided since it will cause poor closing of the rubber about the cannula used for drawing off the plasma, thus breaking the closed system. After sealing the bottle, mix the blood well for one minute.

Storage and centrifugation of the blood are carried out as described in the sections on these subjects, beginning on pages 366 and 367. The equipment necessary (centrifuge, torsion balance, and refrigerator) are described on page 364.

ASPIRATION INTO THE POOLING BOTTLE

Pooling Apparatus. Drawing off, pooling, and distribution into individual containers by a closed system may be accomplished with the apparatus shown in the figure below. Essentially, this

Apparatus for pooling, filtration and distribution of plasma



consists of a pooling bottle *1*, an aspirating cannula *2*, and a distributing cannula *3*, assembled as a single unit. The apparatus is steam sterilized and will effectively safeguard the sterility of the plasma. Before sterilization, about 5 ml. of freshly prepared distilled water is placed in the bottle *1*. The pooling bottle should be of a capacity proportionate to the number of bleedings.

The bottle is closed with a three hole rubber stopper *4*. Through one hole passes a piece of glass tubing *5*, 100 mm. long and 7 mm. in outside diameter. The lower extremity of this tube is flanged, and to it is solidly tied a bag *6* which acts as a filter. This bag is made of four layers of 40 mesh gauze and should be at least 5 cm. long. After construction of the filter, it is boiled for five minutes in freshly distilled water, rinsed once or twice, and rapidly air-dried. The

tube *5* is connected by means of transparent amber rubber tubing *7*, about 60 cm. long, to the aspirating cannula *2*. Through the second hole passes a glass tube *8*, 7 cm. long and 7 mm. in outside diameter. The ends are slightly constricted by flaming, and the lumen is filled with cotton. This tube acts as an air filter; to it is attached the rubber hose *9* connecting the bottle with the vacuum pump. Through the third hole passes a glass tube *10*, 100 mm. long and 7 mm. in outside diameter. The lower extremity is connected by a short piece of rubber tubing *11* to a long glass tube *12* which reaches to the bottom of the pooling bottle. The lower extremity of this tube should be slightly bent and drawn so as to reach the angle that the bottom of the bottle forms with the sides. The upper extremity of the glass tube *10* is connected by transparent amber rubber tubing *13*, approximately 60 cm. long, with the distributing cannula *3*.

The plasma is withdrawn from the erythrocyte layer through the large bore aspirating cannula *2* which perforates one of the rubber tubes of the donor bottle. The rubber tubes *E* and *G* stretch across tubes *B* and *C* to form perforable membranes. The cannula is 15 inches (37.5 cm.) long, and 13 gauge, with a hose hub. The cannula should terminate with an end, sharply beveled, and with the opening 4 mm. from the end (p. 365). The cannula is shielded by a rubber sleeve and a glass bell. The sleeve is made of thin, flat black rubber tubing, outside diameter 1 inch (2.5 cm.), commercially known as Gooch crucible tubing. The glass bell is made of a piece of Pyrex glass tubing, 9 cm. long with an outside diameter of 1.5 cm. The upper end is flanged to allow firm attachment to the rubber sleeve. There are two constrictions dividing the bell into approximate thirds where the lumen is reduced so as barely to permit passage of the cannula. This arrangement centers the cannula and keeps it from touching the sides of the open end.

To assemble the component parts of the aspirating cannula, the hub of the cannula is first pushed through a No. 2 rubber stopper and then attached to the rubber tube leading to the pooling bottle. Next the protecting rubber sleeve is slipped over the cannula and rubber stopper and the proximal end tied firmly around the hub. The distal end of the sleeve is tied to the glass bell. The length of the sleeve is such that the opening of the bell extends beyond the tip of the cannula about 10 to 20 mm. The open end of the bell is loosely stoppered with cotton and covered with a paper cap and is then ready for sterilization.

An air filter is employed with this apparatus, as with the vacuum-type equipment. Its preparation is described on page 365.

The distributing cannula *3* is similar to the one used for aspirating, but it is shorter and the opening is at the tip. The length of the cannula and the size of the glass bell vary according to the size and shape of the final container. For the reusable 400 ml. bottle, the cannula should be 5 inches (12.5 cm.) in length. The glass bell has a diameter of 3.5 cm. at its base and a depth of 4.5 cm. A protective sleeve of thin rubber tubing covers the upper two thirds of the cannula. Its lower end is tied to the bell, and the upper end slips over the lower part of the hub of the cannula. The upper part of the hub is connected to the rubber tube *13*. The protective sleeve is of appropriate length to keep the tip of the cannula high within the bell. The thin wall of the sleeve folds up and easily allows shortening as the cannula is pushed through the stopper of the final container. The entire aspirating and distributing apparatus is wrapped as a unit for sterilization.

Pooling Procedure. A discussion of pooled plasma, the pool size, and the addition of dextrose is found on pages 373 to 374. To draw off the plasma, proceed as follows:

1. Provide the tubes marked *7*, *9*, and *13* in the figure on page 381 with screw clamps.
2. Attach a suction bulb or a vacuum pump to the air filter *8* through tube *9*.
3. Prepare carefully the heads of the blood bottles with iodine and alcohol as described on page 374.
4. Prepare similarly the diaphragms formed by rubber tubes *G* and *E* stretched over the tubes *B* and *C*.
5. *Unwrap and unstopper the bell protecting the aspirating* cannula 2 and place it over the rubber diaphragm *E* or *G*. The cannula, protected by the rubber sleeve, is then grasped near the hub and pushed down into the blood bottle until it is about 3 cm. below the surface of the plasma.
6. Push an air vent through the diaphragm of the other tube to allow entry of filtered air to replace the plasma being drawn off.
7. Release the screw clamp on the tube marked 7, and allow the plasma to be sucked into the pooling bottle. Aspiration is carried out until all but a thin layer of plasma is removed. Care must be employed to avoid stirring up the red cell layer. The plasma which is ordinarily left over the cells usually forms a layer about 0.5 to 1 cm. thick (see step 8, p. 375 for use of artificial illumination).
8. When the aspiration is finished, close the screw clamp, and raise the cannula back into the sleeve so that the tip is well within the bell, flame the mouth of the bell and repeat the entire pro-

cedure with the next bottle, until all plasma has been drawn off.

Addition of a Bacteriostatic Agent. This is not recommended (p. 387).

Sterility Test on the Pool. When the desired number of specimens are pooled, close the screw clamps of tubes *7* and *9*, and mix the plasma and dextrose thoroughly by rotating the pool bottle gently. The pool is cultured in the manner described in the section beginning on page 375, except that the now partly used aspirating and distributing unit in this type of apparatus does not lend itself to closure for the recommended twenty-four to forty-eight hour storage period prior to culture. *This cannot be done without breaking the closed system, which is permissible only in a closed sterile room* (p. 409).

To withdraw the desired samples of plasma, tube *9* is detached from *8*, which acts as a filter. Carefully unwrap the mouth of the distributing glass bell; apply it over the mouth of the culture tube or the iodine and alcohol-cleaned perforable rubber stopper of a tube or bottle of culture medium and lower the distributing cannula, perforating the rubber diaphragm (vacuum-type culture tubes or bottles must be used, p. 371). Now release the screw clamp of tube *13* and introduce the proper amount of the citrated plasma into the medium; withdraw the cannula and repeat with three other tubes or bottles containing thioglycollate medium. The details of preparation of the culture tubes or bottles, the amounts of plasma and culture medium, and the handling of the cultures are given in steps 4, 5, 6, and 7 on page 376.

FILLING OF FINAL CONTAINERS FROM THE POOL

The Final Container. The recommended reusable bottle is cylindrical, of low solubility glass blown in a mold, and of a capacity of a little over 400 ml. The body is 72 mm. outside diameter, with a length of 157 mm.; the neck is 22 mm. long, with a tapered bore 12 mm. inside diameter. The thickness of the walls is 2.5 mm. The bottle should weigh not less than 210 gm. A heavy lip allows tight fitting of a hooded amber rubber stopper.

After suitable cleaning, about 1 ml. of freshly distilled water is introduced into the bottle, and the mouth closed with a hooded rubber stopper. A vacuum is then formed by means of a thin needle, introduced through the solid portion of the stopper, connected to a good water pump. The stopper is then covered with a cap of thick paper securely fastened to the neck of the bottle, and the whole is steam-sterilized. The small amount of water in the bottles permits the formation of steam, necessary to proper sterilization. The vacuum lessens the chance that the stoppers will

be blown off during sterilization, and makes possible the introduction of plasma into the bottle by a closed method.

Procedure. With the employment of this type of reusable equipment, the pool should be distributed into the final containers immediately after obtaining the cultures.

1. Let the screw clamp of tube *7* remain closed. (Tube *9* is still detached from tube *8*, which acts as a filter).
2. Make ready the final container by removing the paper cap protecting the sterile perforable rubber stopper. Clean it carefully with iodine and alcohol.
3. Grasp the hub of the distributing cannula and insert it through the sterile rubber stopper.
4. Release the clamp of tube *13* and allow the container to fill. Close the clamp when sufficient plasma has been transferred. The preparation of these bottles provides that a vacuum be drawn prior to sterilization.
5. Prepare and fill additional containers by the same procedure.
6. In filling the final containers, a pilot bottle should be prepared and cultured as directed in the appropriate sections on page 377.

Release of the Pool for Use. Plasma prepared by the method just presented is released and stored in the same manner as described on pages 377 and 378.

CITRATE SEDIMENTATION METHODS

These methods utilize gravity to achieve the necessary separation of the plasma for aspiration. As a general rule, they are employed only to recover the plasma from outdated stored blood in the operation of a whole blood bank. However, where a suitable centrifuge is not available, they provide a satisfactory means for the preparation of plasma.

Preservative Solutions

To carry out the preparation of plasma by this method, any of the preservative solutions described in Chapter 13 may be employed. To obtain the greatest yield of plasma and the maximum clarity, the blood should be collected in one of the dextrose preservative solutions and allowed to sediment for fourteen days. The yield after this storage period is only slightly less than that obtained by centrifugation. However, when only sodium citrate solution is employed for the sedimentation method the limit of storage is only seven days and the yield is about 30 per cent less.

As indicated previously, *all the preservative solutions containing dextrose* will permit the optimum two week sedimentation period, the principal difference between the plasma obtained with the several solutions being the degree of dilution. The clinical use of dilute plasma has been discussed on page 351.

When only sodium citrate solution is employed, it has been found satisfactory to extend the five day period for use as whole blood to seven days for the aspiration of plasma. The yield and clarity of the plasma are much improved, as a rule, by the two additional days of sedimentation and, although red cell breakdown has occurred to some extent, the free hemoglobin has not yet had time to diffuse significantly into the plasma layer.

Centrifugation of Stored Blood

This is not recommended if the blood has been stored longer than three days in citrate solution since it usually results in an increase in the free hemoglobin in the plasma, and it is not necessary if adequate gravity sedimentation is carried out as just described. Centrifugation may, however, be employed up to ten days if the blood has been stored in a dextrose preservative solution.

Aspiration of the Plasma

When the dextrose-containing preservatives are used, especially those of larger volume, it has not been customary to pool the plasma obtained (see *Dilute Plasma*, p. 351). Aspiration, culture, storage and use of the plasma, are carried out exactly as has been described for unpooled plasma prepared by the centrifuge method, except that it is unnecessary to add dextrose because the fibrin will have precipitated during the two week storage period (p. 350).

When plasma is being prepared from blood stored in citrate solution it is desirable to pool the plasma in 1000 or 2000 ml. lots if the product obtained is to be comparable in appearance to that resulting from the centrifuge method. Aspiration, culture, storage, and use are carried out as previously described for pooled plasma prepared by the centrifuge method with *one exception:* the plasma pool is not immediately distributed to final containers, but is allowed to stand at room temperature for seven to ten days. This step is important because it permits the additional desirable period of sedimentation which will result in a product comparable in clarity to that obtained by centrifugation. However, pooling and an added sedimentation period are not by any means essential. Unpooled plasma may be prepared by this method. Clarity has no bearing on clinical efficacy.

GENERAL CONSIDERATIONS IN PLASMA PREPARATION

Preparation of Concentrated Liquid Plasma

Two methods have been described for this purpose: one by evaporation of water from the plasma when it is placed in a cellophane container; the other, the partial separation of the water from the proteins by repeated freezing and thawing. There are several techniques for the first method, which is not recommended (p. 352), and the latter technique is described on page 398.

Filtration in the Preparation of Liquid Plasma

In some laboratories[10,11,12] it has been the practice to filter liquid plasma for one or both of the following reasons: to clarify the plasma by removing all particulate matter, usually lipoids and precipitated fibrin; and to remove any chance bacterial contaminants. Davenport[10] has described a method using the Berkfeld filter, and Anderson[11] has described a method employing Seitz filtration which is used in the Michigan State Department of Health.

Filtration by either method is difficult to carry out, time-consuming, and adds to the cost of the plasma. In the author's opinion filtration does not add materially to the usefulness, safety, or appearance of plasma if the donors have been properly prepared for bleeding and the plasma processed as described.

While filtration is not recommended in the preparation of plasma, all plasma must be filtered at the time of administration to the patient.

Addition of a Bacteriostatic Agent

It has long been the accepted practice to add a bacteriostatic agent to any biologic product prepared for parenteral use. Before World War II, it was considered essential to add such an agent to blood plasma. However, the investigations conducted during the early war years showed this to be both unnecessary and undesirable. It was found that plasma could be prepared as a sterile product and stored in sealed containers without danger of contamination. The mercurial salts employed for the purpose were potentially dangerous since toxic amounts could be given to patients requiring massive transfusion, particularly if any kidney damage were present. Furthermore, it was found that these mercurials were quite ineffective in preventing the growth of all but a very few bacteria in the concentrations permissible for use, since the mercury becomes bound to the plasma protein and is essentially inactivated as far as bacteriostatic action is concerned. As a result, the Sub-

committee on Blood Substitutes of the National Research Council recommended that the use of a bacteriostatic agent in blood plasma be no longer a required procedure. It was officially made optional for hospital practice in the Office of Civilian Defense Manual, "The Operation of a Hospital Transfusion Service," published in March 1944,[16] and has been made optional for commercial firms by the National Institute of Health. The current regulation of the latter is as follows:*

The Preservative. There is no preservative presently available which is bactericidal to all probable contaminants when used in a concentration which will not be dangerously toxic in the maximum human dose of plasma or serum. Therefore, the addition of a preservative is not specifically recommended or required at this time. Two methods of maintaining sterility are recognized as acceptable.

Method A. No preservative is added. Reliance is placed entirely on good blood collection and processing technique and on rigid sterility tests on the plasma pool or serum and followed by similar tests on final container samples after storage.

Method B. This method relies upon the same standards of blood collection and processing as required under Method A. In addition, a mercurial preservative may be added provided the label bears a caution statement. Not more than 1:10,000 sodium ethylmercurithiosalicylate or 1:15,000 phenyl mercuric borate may be added. The caution statement on the label shall be placed in a prominent position and is worded as follows: '*Caution.* The contents contain—(state kind and amount of mercurial used)—. Do not administer more than 2000 cc. per 50 kilograms of body weight in any twenty-four hour period.'

LABELING OF PLASMA CONTAINERS

It is essential that each bottle of plasma be properly labeled: (1) so that the clinician may know exactly what he is giving to the patient, and (2) so that each bottle is properly identified as to source for the purposes of follow-up studies which may be required because of untoward reactions, or for other reasons.

The label of the plasma bottle should contain the following information:

1. Name and address of the laboratory preparing the plasma.
2. Lot number or pool number. The laboratory record should identify the blood collections (donors) making up each lot of plasma.
3. Date prepared.
4. Amount and kind of diluent added.
5. Amount of original plasma. Original plasma is defined as

* Minimum Requirements Filtered Normal Human Plasma, Filtered Normal Human Serum National Institute of Health, U S Public Health Service, Bethesda, Md., January 10, 1946.

the liquid portion of the blood as it comes from the veins of the donor, before dilution with the anticoagulant, for example: 350 ml. of citrated plasma is obtained from 550 ml. of blood. If 50 ml. of anticoagulant has been added in collecting the blood, the amount of original plasma is considered to be 300 ml. In other words, original plasma equals the total amount of citrated plasma minus the amount of sodium citrate or other solution used in collecting the blood and processing the plasma.

6. Total protein content of the plasma preparation. A statement of this is optional. However, each laboratory should determine its average standard for the method used.
7. Amount and kind of preservative, if added.
8. Dates and results of cultures.

THE ADMINISTRATION OF LIQUID PLASMA

Liquid blood plasma, prepared as described in this chapter and stored at room temperature, is always available for immediate administration. To prepare it for use, the covering is removed from the stopper; the top of the stopper is cleaned with an alcohol sponge; the vacuum is released by puncturing the rubber diaphragm covering the air tube; the filter set for administration is inserted in the position indicated by the manufacturer; and, with the tubing clamp shut, the bottle is inverted and suspended above the patient. The filter and tubing of the administration set are then allowed to fill with plasma, replacing all air, as is customary in intravenous therapy; a suitable vein is selected, the skin area prepared, the needle is inserted into vein, and the infusion is begun. Ordinarily, the rate of administration should not exceed 10 ml. per minute. A more rapid rate may be necessary in the treatment of severe shock, as discussed in Chapter 3.

Blood plasma must always be filtered at the time of administration and must not be heated before or during administration. A filter of 100 to 200 mesh per square inch is adequate for this purpose (p. 393). As is the case with whole blood, the heating of plasma cannot readily be controlled; it may result in denaturation of the protein, thereby producing severe reactions, and it is not necessary from the therapeutic standpoint (p. 298).

SELECTIONS FROM "MIN[illegible] TERED NORMAL HUMAN PLASMA, [illegible] SERUM," NATIONAL INSTIT[illegible] 10, 1946*

The Bleeding Equipment. The apparatus used for the removal of the blood and the receiving unit shall be chemically clean, sterile, and shall be free of pyrogenic substances.

* These requirements are mandatory only for the preparation of plasma which is to be sold in interstate commerce.

committee on Blood Substitutes of the National Research Council recommended that the use of a bacteriostatic agent in blood plasma be no longer a required procedure. It was officially made optional for hospital practice in the Office of Civilian Defense Manual, "The Operation of a Hospital Transfusion Service," published in March 1944,[16] and has been made optional for commercial firms by the National Institute of Health. The current regulation of the latter is as follows:*

The Preservative. There is no preservative presently available which is bactericidal to all probable contaminants when used in a concentration which will not be dangerously toxic in the maximum human dose of plasma or serum. Therefore, the addition of a preservative is not specifically recommended or required at this time. Two methods of maintaining sterility are recognized as acceptable.

Method A. No preservative is added. Reliance is placed entirely on good blood collection and processing technique and on rigid sterility tests on the plasma pool or serum and followed by similar tests on final container samples after storage.

Method B. This method relies upon the same standards of blood collection and processing as required under Method A. In addition, a mercurial preservative may be added provided the label bears a caution statement. Not more than 1·10,000 sodium ethylmercurithiosalicylate or 1:15,000 phenyl mercuric borate may be added. The caution statement on the label shall be placed in a prominent position and is worded as follows: '*Caution.* The contents contain—(state kind and amount of mercurial used)—. Do not administer more than 2000 cc. per 50 kilograms of body weight in any twenty-four hour period.'

LABELING OF PLASMA CONTAINERS

It is essential that each bottle of plasma be properly labeled: (1) so that the clinician may know exactly what he is giving to the patient, and (2) so that each bottle is properly identified as to source for the purposes of follow-up studies which may be required because of untoward reactions, or for other reasons.

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2. Lot number or pool number. The laboratory record should identify the blood collections (donors) making up each lot of plasma.
3. Date prepared.
4. Amount and kind of diluent added.
5. Amount of original plasma. Original plasma is defined as

* Minimum Requirements Filtered Normal Human Plasma, Filtered Normal Human Serum. National Institute of Health, U S Public Health Service, Bethesda, Md., January 10, 1946.

which a subsequent rise following the injection of the test material is calculated. Warm the product to be tested to approximately 37° C. and inject intravenously, through an ear vein, 10 ml. per kg. of rabbit within fifteen minutes subsequent to the control temperature reading on the day of the test. Record the temperature one hour subsequent to the injection and each hour thereafter until three recordings have been made. Syringes and needles used for these injections must have been treated to render them pyrogen-free by heating in a muffle furnace at 250° C. for not less than thirty minutes. Three rabbits shall be used for each test and the test shall be considered positive if two or three animals show an individual rise in temperature of 0.6° C. or more above the normal established for each of these animals. If only one animal shows a temperature rise of 0.6° C or more, or if the sum of the temperature rises of the three animals exceeds 1.4° C., the test must be repeated on five additional rabbits. The test shall be considered positive if two or more of the group of five rabbits show an individual rise in temperature of 0.6° C. or more above the normal established for these animals.

Limits of Test. The present pyrogen test is designed for products which can be tolerated by the test animal in a dose of 10 ml. per kg. With products requiring the use of test doses of different volume, the individual product minimum requirements will specify the test dose to be used.

Interpretation of the Serologic Test

An acceptable serologic test shall be one which is acceptable to the Chief, Division of Venereal Diseases, United States Public Health Service.

Any blood showing a 3+ or 4+ reaction shall be considered as unsatisfactory and the blood must not be processed.

Any blood showing a plus-minus, 1+ or 2+ reaction shall be checked by another method, preferably a complement fixation test. If the same degree of reaction, or less, is obtained, the blood shall be considered satisfactory. However, if the retest method is a complement fixation test, then a 2+ reaction shall indicate an unsatisfactory blood and the blood must not be processed.

Methods for the Determination of Hemoglobin

For an accurate quantitative determination of the hemoglobin content of the pooled plasma or serum, either of the following methods may be employed, or one equally accurate:

Ham, T. H.: Arch. Int. Med., 64:1271, (Dec.) 1939.

Bing, F. C., and Baker, R. W.: J. Biol. Chem., 92:589, 1931

In the absence of proper facilities for doing an accurate quantitative hemoglobin test the determination shall be considered sufficiently accurate if a colorimetric comparison be made with a standard prepared from hemolyzed blood from a person whose blood hemoglobin content has been determined previously in terms of grams of hemoglobin. The standard is prepared as a dilution of hemolyzed blood in plasma which is free of all

The Serological Test. An acceptable serological test for syphilis shall be made in a qualified laboratory on a specimen of blood taken from the donor at the time of bleeding and the blood shall not be used for the production of normal human plasma or serum unless the result of the test is negative.

A Permanent Record of Each Donor. A permanent record shall be kept of the name, sex, age, and address of each donor and a similar record shall be kept of each pool indicating its lot number and the name of the individual donors comprising the pool. Each final container shall bear a label as directed. . . . . .

The Pyrogen Test

Test Animal. Use healthy rabbits weighing 1500 gm. or more which have been maintained for at least one week on a uniform unrestricted diet and have not lost weight during this period. Use a clinical rectal thermometer and test it to determine the time required to reach maximum temperature. Other recording devices of equal sensitivity are acceptable. Animals which have been used for previous pyrogen tests may be used for subsequent tests after a rest period of not less than two days. In testing allergen-containing materials the test animal shall not be used more than once with the same allergen. If the animals have not been used for tests during the previous two weeks, take four rectal temperature readings on each of the animals at two hour intervals one to three days before use. Insert the thermometer or other recording device beyond the internal sphincter, and allow it to remain a sufficient time to reach maximum temperature, as determined above, before the reading is recorded. Do not use in the test those animals with a temperature in excess of 39.8° C. House test animals in individual cages protected from disturbances likely to cause excitement. Exercise particular care to avoid exciting the animals on the day of taking the control temperatures and on the test day. Maintain the animals in an environment of uniform temperature (±5° C.) for at least forty-eight hours prior to and during the test period. Preferably they should be in quarters maintained at constant temperature and humidity.

Conduct of Tests. Perform the pyrogen test in a room in which the temperature and the humidity are maintained at the same level as that of the room in which the animals are housed for the test. During the test, the animal may be restrained in a suitable type of holder. Withhold food from any animal used, beginning one hour before the first temperature reading, and permit no food until the day's record is completed. Access to water *may* be allowed. On the day of the test take a control temperature reading prior to beginning the injections. However, a period of not more than fifteen minutes should elapse after the removal of the animal from the cage to the time of taking the control temperature if the animal is to be restrained in a holder. Animals may be used for the test provided the control temperature reading taken on the day of the test does not fall below 38.9° C. and does not exceed 39.8° C. The reading taken on the day of the test constitutes the normal temperature of the test animal from

agar. Store at 15° to 30° C. (preferably 20° to 30° C. as low temperature increases absorption of oxygen from the atmosphere); avoid excessive light. If more than 30 per cent of the uppermost portion of the medium has changed to a pinkish color, it is unsuitable for use. Under such circumstances one reheating in a boiling water or steam bath is permissible in order to drive off the absorbed oxygen.

5. Growth-promoting Quality of Medium. It is recommended that each lot of medium be tested for its growth-promoting and oxidation-reduction qualities. For this purpose use one or more bacteria that are exacting in growth requirements. At the end of the incubation period used for the sterility test (seven days) less than 60 per cent of the medium in each tube shall have changed color.

6. Type of Container for Culturing. This medium permits the growth of both aerobic and anaerobic organisms in the same open container. The test tube of choice is one which measures 20 by 150 mm. into which is placed 15 ml. of culture medium. This will provide adequate medium for inoculum up to 3 ml. and adequate thioglycollate to inactivate a mercurial preservative when present in the inoculum in not more than a 1:10,000 dilution. Other preservatives will need to be inactivated by adequate dilution unless an effective inactivator is available and used. With a large volume of inoculum, cylindrical or square bottles having approximately the same ratio of surface exposed to volume of medium as mentioned above are recommended. The inoculum must be mixed thoroughly into the medium in all instances because of the viscosity of the medium. Likewise, the contents of the inoculated tubes should be re-mixed at the time of making the first, or forty-eight hour reading in order to distribute the growth more widely through the medium This will insure a more accurate final reading.

A Filter Adequate for Removal of Particulate Matter

A filter for use in a pooling or transfusion set is considered adequate provided it does not cause critical slowing of the flow and is capable of removing particulate matter of a size potentially dangerous to the patient through intravenous administration. The porosity and screening qualities of the filter should be not less than stainless steel or monel metal wire cloth of 100 to 200 mesh per square inch. The capacity of the filter must be sufficient to accommodate the entire contents of a final container of the product. Experience has shown that a cylindrical stainless steel filter of 100 to 200 mesh with one end closed and measuring 4 cm. in length and 4 mm. in diameter has the capacity to filter 600 ml. of a properly processed human plasma without critically slowing the rate of flow.

REFERENCES

1 Tatum, W. L , Elliott, J , and Nesset, N : A technique for the preparation of a substitute for whole blood adaptable for use during war conditions. Mil. Surgeon 85:481, 1939

visible trace of hemoglobin color. Hemolysis of the blood is effected by first diluting the blood 1 to 20 with distilled water and waiting until hemolysis is complete.

The blood hemoglobin in the person serving as the source of hemoglobin supply shall be determined by a method at least as accurate as can be made by a Sahli apparatus equipped with permanent color standards. These color standards shall have been calibrated during the course of preparation by the manufacturer, using the Van Slyke oxygen capacity method, the Wong iron method, or a method recognized as the equivalent. (Am. J. Clin. Path. 3:85,1933; 4:354, 1934.)

Fluid Thioglycollate Medium for the Sterility Test

Full details of the formula and method of preparing the fluid thioglycollate medium and its broth modification are given in the National Institute of Health Circular: "Culture Media for the Sterility Test." For convenience, sections 3, 4, 5, and 6 of that circular are included in this appendix. The complete fluid thioglycollate medium in the dehydrated form may be purchased from at least two manufacturers.

3. Fluid Thioglycollate Medium

1-cystine (Reagent)	0.75 gm.
Sodium chloride	2.5 gm.
Dextrose ($C_6H_{12}O_6.H_2O$)	5.5 gm.
Granular agar (less than 15% moisture by weight)	0.75 gm.
Water soluble extract of yeast	5 gm.
Pancreatic digest of casein	15 gm.
Distilled water	1000 ml.
Sodium thioglycollate or	0.5 gm.
Thioglycollic acid	0.3 ml.
0.10% solution of resazurin (freshly prepared)	1 ml.

Some difficulty may be experienced in getting the 1-cystine into solution. (Hydrochloric acid may not be used as an aid in dissolving.) One method is to mix in a mortar all of the dry ingredients except the sodium thioglycollate (or the thioglycollic acid) in the order given in the table, thoroughly mixing each as it is added. Then stir in a portion of the water (previously heated) transfer to a suitable container, add the remainder of the water and complete the solution by heating in a boiling water or steam bath. Then add the sodium thioglycollate or thioglycollic acid. Irrespective of the method used, it is preferable to add the sodium thioglycollate or thioglycollic acid after the preliminary heating. Adjust the reaction with sodium hydroxide to such a point as experience shows will result in a pH of 7.1 ± 0.1 in the completed and sterilized medium. Reheat, but do not boil, and filter (only if needed for clarification) through a moistened paper filter, then add the resazurin solution. Distribute into final containers and sterilize in the autoclave for 18 to 20 minutes at 121° C.

4. Storage of the Medium. After removal of the final container of medium from the autoclave, cool promptly to 25° C. in order to set the

agar. Store at 15° to 30° C. (preferably 20° to 30° C. as low temperature increases absorption of oxygen from the atmosphere); avoid excessive light. If more than 30 per cent of the uppermost portion of the medium has changed to a pinkish color, it is unsuitable for use. Under such circumstances one reheating in a boiling water or steam bath is permissible in order to drive off the absorbed oxygen.

5. Growth-promoting Quality of Medium. It is recommended that each lot of medium be tested for its growth-promoting and oxidation-reduction qualities. For this purpose use one or more bacteria that are exacting in growth requirements. At the end of the incubation period used for the sterility test (seven days) less than 60 per cent of the medium in each tube shall have changed color.

6. Type of Container for Culturing. This medium permits the growth of both aerobic and anaerobic organisms in the same open container. The test tube of choice is one which measures 20 by 150 mm. into which is placed 15 ml. of culture medium. This will provide adequate medium for inoculum up to 3 ml. and adequate thioglycollate to inactivate a mercurial preservative when present in the inoculum in not more than a 1:10,000 dilution. Other preservatives will need to be inactivated by adequate dilution unless an effective inactivator is available and used. With a large volume of inoculum, cylindrical or square bottles having approximately the same ratio of surface exposed to volume of medium as mentioned above are recommended. The inoculum must be mixed thoroughly into the medium in all instances because of the viscosity of the medium. Likewise, the contents of the inoculated tubes should be re-mixed at the time of making the first, or forty-eight hour reading in order to distribute the growth more widely through the medium. This will insure a more accurate final reading.

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A filter for use in a pooling or transfusion set is considered adequate provided it does not cause critical slowing of the flow and is capable of removing particulate matter of a size potentially dangerous to the patient through intravenous administration. The porosity and screening qualities of the filter should be not less than stainless steel or monel metal wire cloth of 100 to 200 mesh per square inch. The capacity of the filter must be sufficient to accommodate the entire contents of a final container of the product. Experience has shown that a cylindrical stainless steel filter of 100 to 200 mesh with one end closed and measuring 4 cm. in length and 4 mm in diameter has the capacity to filter 600 ml. of a properly processed human plasma without critically slowing the rate of flow.

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CHAPTER 17

Frozen Plasma

By JOHN B. ALSEVER

The preparation and administration of *frozen plasma* is not technically difficult but requires special equipment and therefore is more expensive than processing the liquid form. The plasma is prepared exactly as described in Chapter 16 and is then frozen.[1,2,4] The freezing of blood plasma, within seventy-two hours of the time of bleeding the donor, stabilizes all protein fractions for long storage essentially as in the fresh state. The advantages and disadvantages of the method and the clinical uses of frozen plasma are discussed on pages 12 and 349.

EQUIPMENT

The special equipment required for the preparation, storage and use of frozen plasma consists of a refrigerator in which liquid plasma can be rapidly frozen and stored at low temperature, and facilities for rapid thawing of the plasma for administration.

Refrigeration. Freezing and storage can be accomplished in certain frozen food refrigerators now on the market, which are fitted with special small compartments for rapid freezing, if large additional storage space is not required. A 25 cu. ft. box will hold approximately five hundred 300 ml. units of plasma. The box must be capable of maintaining a temperature of at least −20° C. The quick freezing compartment of such a refrigerator provides for a rapid transfer of heat, either by means of a fan to circulate the air in the small compartment or an arrangement

of the refrigerating coils and bottle holders which permits direct contact with the plasma bottles. Quick freezing may be accomplished in any low temperature refrigerator, however, by immersing the bottles in a container filled with a fluid, such as alcohol, which will not freeze at −20° C.

Water Bath. Rapid thawing can best be accomplished by one of the deep, electrically heated water baths which is thermostatically controlled to maintain a temperature of 37° C., with a water circulator and a high heating capacity especially designed for this purpose.

PREPARATION OF PLASMA BEFORE FREEZING

The liquid plasma is prepared in containers and cultured by any one of the methods described in Chapter 16. However, if a maximum content of prothrombin and complement are desired, the centrifuge method should be employed, the entire process completed, and the plasma brought to the frozen state within seventy-two hours. In addition, the whole blood and the aspirated plasma should be held constantly at 4° to 10° C. to minimize the loss of these labile components.

Sterility. If the frozen plasma is prepared in the fresh state as described above, the preculture storage of the pool (p. 375) cannot be carried out and the final product will be slightly cloudy from the precipitation of small amounts of fibrin. Since storage at low temperature will act as an efficient bacteriostatic, a chance contaminant will not be dangerous if the plasma is not allowed to remain at room temperature before use after it has been thawed. The pilot bottle may, if desired, be frozen for seven to ten days before culturing so that the final sterility test may be carried out with plasma which has been frozen. It is recommended, however, that the twenty-four hour room temperature storage of the pilot bottle be carried out before the sample for culture is removed, since this will more adequately demonstrate the sterility of the product. If this modified technique is employed for culturing the pool, the final product may not be sterile. This is not important, however, if the plasma is kept frozen and is thawed *just before* its administration to the patient. Maintenance in the frozen state will almost invariably prevent bacterial growth and there will be insufficient time in the liquid state for a chance contaminant to multiply. Should it be desirable to keep the plasma longer than a few hours in the liquid state after thawing, it is recommended that maximum freshness of the product be sacrificed in favor of the sterility procedures required for liquid plasma (pp. 370 and 375).

The antibody content will not, of course, be affected by this technique.

Addition of Dextrose. It is not necessary to add dextrose to the plasma pool unless storage in the liquid state for a time after thawing is desired. The addition of dextrose will not prevent rapid freezing of the product in a satisfactory manner under the conditions described.

Filling of Final Containers. When plasma is to be frozen, the final containers should not be filled more than about three fourths full. This precaution will eliminate for the most part the danger of breakage of the glass as the plasma expands during freezing.

FREEZING THE PLASMA

If the plasma has been prepared for storage in the fresh state it must be frozen promptly after the final containers are filled. Otherwise, liquid plasma may be frozen at any desired or convenient time. One of the mechanisms for quick freezing described under *Equipment* should be employed. The plasma should be completely frozen within four to six hours.

It is preferable to place the bottles so they are slightly tilted to provide a somewhat larger surface area to accommodate the expansion of the liquid plasma during freezing. The angle must not be great enough to bring the plasma into contact with the stopper of the container. Some freezing cabinets, especially built for this purpose, provide a sloping freezing surface, but a simple wooden or wire frame may be constructed to hold the bottles at the proper angle in any refrigerator compartment. If the equipment is available, the plasma may be shell frozen (p. 406). This has some advantage but is a more expensive method and is essential only in the preparation of dried plasma. The advantages of shell freezing are: the plasma may be frozen very quickly, the time depending on the temperature employed, and complete thawing occurs much more rapidly in the water bath.

STORAGE OF FROZEN PLASMA

After freezing, the plasma must be stored in a refrigerator cabinet which will maintain a temperature of −20° C. or lower, with less than 2 or 3 degrees of variation. If the storage temperature is raised to −15° C., the labile components of plasma will slowly disappear, although the plasma remains frozen. If the product is maintained at or below −20° C., it is quite likely that it may be stored indefinitely. It is known that it will not deteriorate in at least three years.

THAWING OF FROZEN PLASMA

Frozen plasma must be brought rapidly to the liquid state at 37° C. in a water bath of the type described earlier in this chapter, to avoid precipitation of the fibrin during thawing. The desired number of bottles of plasma are removed from the storage cabinet and placed at once in the water bath. The standard size bath for plasma will accommodate four to six containers of 300 ml. size and will thaw the plasma and bring it rapidly to room temperature in twenty to thirty minutes. The containers must be left in the water bath after initial liquefaction of the contents so that the temperature will be brought rapidly past the critical range of 0° to 15° C. in which the fibrin precipitates.

STORAGE AND ADMINISTRATION OF RELIQUEFIED PLASMA

To safely store reliquefied frozen plasma at room temperature two precautions must be taken: (1) dextrose must have been added to stabilize the fibrinogen, and (2) the sterility testing must have been carried out as described for liquid plasma (pp. 369 and 370). If the frozen plasma was not so prepared, it may safely be kept only a short time at room temperature before administration to the patient, preferably less than one hour. It may always be refrozen if it cannot be used after thawing.

Reliquefied plasma is administered to the patient in the same manner as plasma which has been stored in the liquid state (p. 389).

PREPARATION OF CONCENTRATED PLASMA BY REPEATED FREEZING

Antopol and his coworkers[4] described an inexpensive and satisfactory method for the preparation of concentrated plasma. They observed that when frozen plasma is thawed without any agitation there is some separation into layers; the lowermost layer is deep yellow in color and rich in proteins, the uppermost is nearly colorless and almost devoid of proteins. On repeated freezing and thawing, without agitation of the plasma, the separation becomes more distinct. After a bottle of plasma has been frozen and thawed three times, the lower fourth contains protein in a concentration of about 15 gm. per 100 ml. Centrifugation after thawing improves the concentration. After achieving the desired separation, the supernatant plasma, low in protein, is aspirated off and discarded. If it is desired to store the concentrated plasma for future use, it *must be transferred* to a new container, since the thin diaphragm of the stopper will not maintain a seal after puncture by the

aspirating cannula. Concentrated plasma and its uses are discussed on pages 12 and 351.

SELECTED EXCERPTS FROM "MINIMUM REQUIREMENTS"*

Method of Handling Plasma or Serum to be Frozen. Plasma or serum to be processed to the frozen state shall be placed in the freezing compartment immediately after filling. In order to satisfactorily bring the plasma or serum to the frozen state, the properly stoppered containers are placed in a mechanical deep-freeze type of compartment or its equivalent. The temperature of the freezing compartment or device, and its freezing capacity, shall be such that each bottle of liquid plasma or serum placed therein will be brought in its entirety to the frozen state within six hours.

Frozen Plasma or Serum. The expiration date shall not exceed three years from the date of manufacture when kept continuously in the frozen state. . . . The date of manufacture is calculated as the date of bleeding the donor.

Frozen plasma or serum shall be stored continuously at −18° C. or lower until needed.

Frozen plasma or serum shall be thawed in the following manner: The bottle of frozen plasma or serum is placed immediately in a constant temperature water bath provided with circulating water, or its equivalent, and maintained at 37° C. As soon as all of the plasma or serum is melted, and its temperature has reached the specified storage range for liquid plasma or serum, the bottle is removed and stored until used as recommended for liquid plasma or serum.

There is considerable risk involved in shipping frozen plasma or serum and therefore it is not a recommended practice. The use of this product should be restricted to the processing laboratory or its immediate vicinity.

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* From "Minimum Requirements: Filtered Normal Human Plasma, Filtered Normal Human Serum," National Institute of Health, January 10, 1946. These requirements are mandatory only for the preparation of plasma which is to be sold in Interstate commerce.

CHAPTER 18

Dried Plasma

By John B. Alsever

The preparation of dried blood plasma is much more expensive than the production of the liquid or frozen forms. It is technically difficult and requires costly equipment and a well-trained staff. However, the resulting product is stable for at least five years under a wide variety of conditions. The administration of dried blood plasma requires preliminary reconstitution to the liquid state by the addition of the desired amount of sterile pyrogen-free 0.1 per cent citric acid in sterile distilled water. The diluent is usually packaged with the container of dried plasma in the amount required to restore the product to its original volume. Dried plasma goes into solution within a few minutes and is then ready to administer. The advantages and disadvantages of the method and the clinical uses of dried plasma have been discussed on pages 12 and 349.

GENERAL PRINCIPLES OF DRYING FROM THE FROZEN STATE

The drying of blood plasma, or other biologic, from the frozen state permits the process to be carried out without any significant change in the constituents, except for the loss of water, in such a manner as to permit prompt reconstitution to essentially the original condition.[5,6,7] This is possible because the ice has a higher vapor pressure at subzero temperatures than the other constituents and the water is removed by vacuum while the low temperature maintains the integrity of the plasma proteins. In general, the following conditions must be fulfilled to dry blood plasma successfully: (1) the

vacuum must be sufficiently high so that water cannot exist as a liquid; (2) the necessary heat must be supplied to the frozen plasma for the conversion of the water from ice to vapor; (3) the water vapor must have ready egress from the container of frozen plasma and free diffusion to the point of removal; and (4) the water vapor must be rapidly and continuously removed from the system. As has been pointed out in this connection, plasma could be dried instantaneously under ideal conditions (*a*) if the film of frozen plasma were thin enough, (*b*) if the surface for sublimation of the ice and condensation of the water vapor were infinitely large, (*c*) if the temperature were maintained at a constant level, and (*d*) if the vacuum were perfect. The several types of plasma drying apparatus in use today represent various methods of achieving a practical approach to this ideal. A brief description of the principles and techniques commonly employed is given in the following sections.

Vacuum

The drying chamber must be vacuum tight. The connection to the vacuum pump must be of sufficient size and the pump of sufficient capacity to exhaust the air rapidly to 500 microns (0.5 mm.) of mercury pressure or less, to avoid melting of the frozen plasma during the initial phase of the drying cycle. The magnitude of the vacuum determines the temperature at which sublimation of the ice occurs. At a pressure of 500 microns, vacuum sublimation will occur at −31° C.; at 100 microns, −40° C. The vacuum pump should be capable of exhausting the system finally to a vacuum of from 50 to 100 microns.

Condenser

Since the movement of the water vapor from the frozen plasma is by diffusion, the mouths of the plasma containers should be as close as possible to the point of removal of the vapor, which is the condenser. If the vapor is not rapidly removed, the magnitude of the vacuum is decreased until the plasma melts (bottle size and neck opening are important; see requirements on p. 405). When water vapor is being transferred in a low pressure system, there is a vacuum gradient, the vacuum being least in the bottles of frozen plasma and greatest at the condenser. The difference between the two is in direct proportion to the distance involved. The water vapor may be removed from the system by one of four methods:

Oil Pump. The water vapor is taken up by circulating oil which is passed continuously through a centrifuge on the atmospheric side of the system where the water is removed. This method

is slow. A high vacuum cannot be maintained, since it is impossible to remove all the water. The speed of the drying process is, therefore, much reduced over other methods and the drying takes place dangerously close to the melting point of the plasma.

Chemical Desiccants. It is possible to remove the water vapor satisfactorily by the use of such absorbent agents as phosphorus pentoxide, silica gel, and specially prepared calcium sulfate, which can be regenerated (dried) for repeated use. However, the volume of desiccant required is very large and the available surface area for absorption must be quite extensive. The temperature must be kept constant by removal of the heat liberated by absorption of the vapor, and drying ovens must be available for regeneration of the desiccant. As a result the absorption of the water vapor is slow, the desiccant is bulky to handle, and considerable time must be consumed in regeneration of the desiccant to prepare for further use of the apparatus.

Aspirator Condenser. The multiple-stage steam ejector has been a practical method in very large installations. A five-stage steam ejector will exhaust large drying chambers to a vacuum of 100 microns. This method, however, is not possible unless 90 to 150 lbs. (40 to 68 kilograms) of steam pressure is available and large amounts of water at about 25° C. can be used for cooling of the steam, to remove the water vapor and air in the condensing chamber. A large amount of space is required so that it is not a practical method for small units.

Refrigerated Condenser. A cold surface is by far the most efficient method for the removal of water vapor, provided the temperature is low enough, the cold surface is sufficiently large, and the required temperature can be maintained regardless of the amount of water vapor which is condensing. The method is both economical and practical with mechanical refrigeration. A large surface area can be provided for instant freezing. The heat of condensation and crystallization can be removed adequately to maintain a temperature of −30° C. or lower, at which the water vapor pressure is low enough to permit good vacuum.

Ice condensed in vacuum is a good heat conductor. It has been shown that there is about 1° C. difference for each centimeter of ice up to a thickness of 5 cm. Two types of condensers are in common use: (1) a flat or curved surface type, probably to be preferred, as in the Michael Reese machine (p. 412) or the "pig" dryer (p. 414); (2) a system of coils and vapor deflectors (baffles), as in the Strumia machine (p. 409). In the latter type, the design of the condenser presents the problem of preventing the possible building up of ice on the first cold surface in the path of the water

vapor to such an extent as to impede or block the vapor flow to the remainder of the cold surfaces intended for condensation.

HEAT

If heat is not supplied to replace the specific heat of ice sublimation, an equilibrium is established between the frozen plasma and the condenser so that drying ceases. The amount of heat required varies as the drying progresses through the characteristic three stages of a drying cycle (see *Drying*, below). Heat may be supplied by conduction or infra-red radiation.

Conduction. The amount of heat which can be employed is limited since it is absorbed by the thin layer of frozen plasma next to the bottle. Too much heat results in thawing of this layer, especially at the beginning of the drying cycle, with denaturation and fusing of the plasma proteins. The conduction method is used by employing room temperature and/or warmed air in the "pig" dryer (p. 414) and a warm water jacket in the Strumia dryer (p. 409). When air is used, the heat exchange may be accelerated by the use of lamps to increase the air temperature and fans to provide circulation of the warm air. However, caution must be used to avoid excessive heating and, if fans are used, the current of air *must not* be directed on the bottles of frozen plasma.

Infra-Red Radiant Heat. Since infra-red rays penetrate and therefore act on the entire mass of frozen plasma, this source is to be preferred to conduction heating for drying shell-frozen blood plasma. Its use, however, adds considerably to the initial cost of the drying equipment and it is more complex and difficult to maintain, as judged by the early experience with this type of apparatus. Developed by Levinson and Oppenheimer, this equipment employs for each bottle an individual heating unit which operates at 400° to 700° C. Higher temperatures burn the plasma next to the glass, even though the inner layer is still frozen, while lower ranges of temperature are largely absorbed by the glass bottle, resulting in melting of the outer layer of the plasma. Within the temperature range specified, drying takes place in an ideal manner from the inside out, with maximum speed, and results in an excellent product.

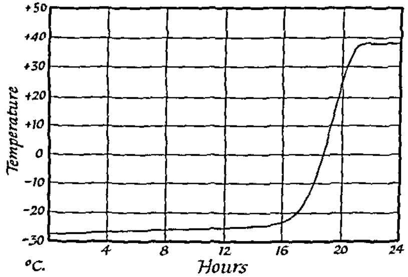
DRYING

The drying cycle of frozen plasma takes place in three stages. A graph of a typical drying cycle is shown on page 404. The time required for each stage depends on the vacuum, the temperature, and the efficiency of the removal of the water vapor in the apparatus being employed.

First Stage. This is the principal drying period in which about

90 per cent of the water is removed. The temperature of the frozen plasma must not be allowed to go above −15° C. during this period. The maintenance of a constant temperature in the frozen plasma indicates that the water vapor is being condensed as rapidly as it is being produced. Excessive heating results in thawing of at least a portion of the plasma and denaturation of the proteins, as well as some fusing of the protein into relatively insoluble particles. *Fused plasma is quite toxic.*

TEMPERATURE CURVE OF A 24-HOUR DRYING CYCLE



Second Stage. In the intermediate drying period about 9 per cent additional water is removed. During this phase, the temperature of the plasma is allowed to rise slowly and gradually to 35° to 40° C. This operation forms the vertical part of the S-shaped curve.

Third Stage. Up to nine tenths of the remaining 1 per cent of water can be removed, depending on the apparatus employed. Relatively high temperatures are safe when the plasma is 99 per cent dry and, as a rule, three to four hours at 45° to 55° C. will suffice to bring the final moisture content to a minimum for the machine used (with infra-red heat, less than 0.1 per cent).

SEALING AND STORING

The bottles of dried plasma must be sealed very rapidly since the dried material is quite hygroscopic. The plasma should either be sealed in vacuum or in an atmosphere of dry nitrogen. Even with nitrogen a vacuum is desirable and facilitates reconstitution. Either method prevents accidental increase in moisture above the safe content for storage and provides an oxygen-free atmosphere within the bottle. Plasma with less than 0.5 per cent moisture will safely

stand a temperature of 80° C. The accepted standards, however, call for less than 1.0 per cent water content of the final product at all times and stipulate that storage shall not normally be above 37° C. (p. 418).

REQUIREMENTS FOR THE GLASS CONTAINER

The bottle employed for the final container of dried plasma must have a low coefficient of expansion and must be free from strains to withstand the sudden temperature change of shell freezing. The glass should be of high silica content; the walls 3 mm., or slightly more, in thickness and quite uniform throughout, to avoid accidental breakage due to the pressure imparted by the freezing plasma. The contour of the bottle should avoid acute angles; a slightly curved bottom and sloping shoulders permit maximum egress of water vapor. The neck of the bottle is as important as *any other factor* in controlling the rate of drying; an aperture 20 mm. in diameter is the smallest compatible with satisfactory and rapid drying. The effect of neck diameter is illustrated by the fact that eight times as much vapor per unit of time will flow through a 20 mm. aperture as compared to a 10 mm. opening.

HYDROGEN ION CONCENTRATION OF DRIED PLASMA

Since the drying process removes gases as well as water from the frozen plasma, the loss of carbon dioxide results in a pH of 8.2 to 9.3 when the plasma is restored to the liquid state with distilled water. This change from the normal pH inactivates the prothrombin. Reconstitution with 0.1 per cent citric acid in distilled water results in a pH of 7.4 to 7.8 and essentially full activity of the prothrombin.[11]

PREPARATION OF PLASMA BEFORE SHELL FREEZING

The containers of liquid plasma may be prepared and cultured by any one of the methods described in Chapter 16. However, if maximum content of prothrombin and complement is desired, the centrifuge method should be employed, the entire process completed, and the plasma brought to the frozen state within seventy-two hours. In addition, the whole blood and the aspirated plasma should be held constantly at 4° to 10° C. to minimize the loss of these labile components.

STERILITY

The plasma pool is cultured immediately after pooling, without the preculture storage of the pool as required for liquid plasma.

The technique is described on pages 375 and 384, depending on whether commercial or reusable equipment is employed for the preparation of the pool.

Pilot Bottle. This is prepared as described on page 377, shell frozen and dried with the rest of the lot. It is then stored for two weeks, reconstituted and cultured as described on page 377.

If a positive test results from either the pool or pilot bottle culture, the lot may still be released for use provided a filled container selected at random from the lot is cultured, in duplicate 25 ml. samples, at both 37° C. and room temperature with negative results.

Addition of Dextrose

The addition of dextrose to the pool is not necessary when plasma is to be dried, although the sugar does not interfere with drying. Dextrose serves no useful purpose in dried plasma since the product is restored to the liquid state only at the time of administration. Reconstituted dried plasma should not be stored at room temperature.

Filling of Final Containers

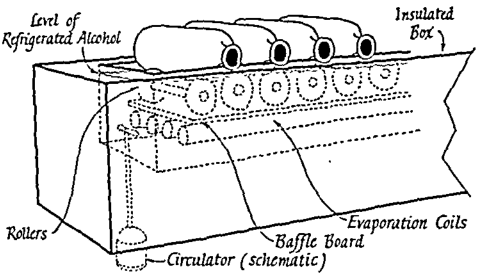
The plasma should be filled into final containers as described for reusable equipment (p. 381), since the filling must be into the special type of container described on page 405, which is closed with a stopper that can be readily removed for drying. The method of aspirating from the pool bottle and taking the pool cultures will depend, of course, on whether commercial vacuum type or reusable equipment is employed up to this point. Meticulous aseptic technique is essential in handling plasma which is to be dried, because the process necessitates the use of an open system. The use of a closed sterile room (p. 409) is desirable in filling of the final containers and essential in handling the plasma during the drying process.

SHELL FREEZING

Special apparatus is required to shell freeze blood plasma. The diagram on page 407 shows one variation of the standard design. This particular freezer is incorporated in the Strumia drier discussed on page 409. It provides a bath of circulating alcohol maintained at −20° to −35° C. by mechanical refrigeration, with cooling coils contained in the base of the apparatus. It is fitted with motor-driven wheels to hold and rotate the bottles of plasma during the freezing operation. The alcohol level is so adjusted as to permit the bottles to be immersed to a depth of 12 mm.

The principal variations in mechanical shell freezers involve (*a*) the temperature employed, and (*b*) the method of preventing plasma from freezing in the neck of the bottle. Shell freezing is usually performed at temperatures of −60° to −72° C. either by use of the cascade type of mechanical refrigeration or by the use of dry ice and alcohol. Such a temperature range avoids any possibility of fractional separation of the plasma by ice crystallization, which may occur when shelling is done at higher temperatures and

APPARATUS FOR SHELL FREEZING



results in an uneven lumpy shell. However, the method of operation to be described essentially eliminates this danger and the utilization of the higher temperature range is more economical, both in initial cost and in maintenance. The most important difference between the two procedures is in the speed of shell freezing. The importance of the size of the diameter of the neck of the plasma bottle has already been emphasized (p. 405). It is evident, therefore, that the plasma must not be allowed to freeze in the neck of the bottle. There are three methods of prevention: (1) The use of a sufficiently large and properly shaped bottle to keep the plasma level below the neck when the bottle is placed in a horizontal position on the shelling apparatus; (2) The location and design of the rotating bottle holders in the freezer to permit two positions; an initial placement of the bottle at a slant which keeps the neck free of plasma until freezing reduces the volume sufficiently so that liquid plasma does not reach the bottle neck, and a final horizontal position to effect an even distribution of the frozen shell. This method requires considerable attention. (3) The installation of an

electrically heated metal collar over the necks of the bottles when they have been placed in the freezer. Two other methods which have been employed for shell freezing are relatively unsatisfactory: (1) hand shelling, because it is tedious and very difficult to obtain an even shell, and (2) spin freezing, which involves an apparatus to hold and rotate the plasma bottles at very high speeds in a cold atmosphere. This is slow and mechanically complex although it produces an excellent shell.

Operation of the Shell Freezer

The technique for the shell freezing of plasma at −20° to −35° C. as described by Strumia,[5] is as follows:

1. The bottles are rotated at ½ to 1 revolution per minute until the plasma has been cooled to about 10° C. This takes from three to ten minutes, depending on the temperature of the plasma and the alcohol bath. The precooling may also be done, before placing the plasma in the sheller, by a few hours of storage at 4° to 10° C. after filling the final containers. In this case essentially no precooling is required in the sheller.
2. Rotation is stopped to permit a thin layer of 3 to 4 mm. of plasma to snap freeze along the dependent portion of the bottle. This takes place in two to four minutes, and as a rule is easily seen.
3. Rotation is started again and the shell freezing goes on at a regular rate to produce an even shell, requiring about seventy-five to eighty-five minutes.
4. The frozen plasma is now stored at −20° C. or below for at least four hours to allow the temperature of the plasma to drop to this level (it is at 0° C. when the shell freezing is complete). This technique, according to Strumia, produces a quality of shell frozen plasma practically identical with the product frozen at −60° to −70° C.

The technique of shelling at −60° to −70° C. with dry ice and alcohol or cascade refrigeration is essentially similar, except that the process is complete within fifteen or twenty minutes. The plasma is precooled as before, rotation is stopped briefly to initiate the shelling, recommenced to produce an even plasma shell as freezing continues. Again, the plasma is stored at −20° C. or below before drying.

Storage of Shell-Frozen Plasma

Shell-frozen plasma must be stored for at least four hours at or below −20°C., as previously described, before drying. However, plasma may be stored safely for any longer period after freezing, if the low temperature is maintained, or it may be thawed at any

time for administration (p. 398). Higher temperatures, even for brief periods, may result in distortion of the shell and subsequent drying will be unsatisfactory.

THE CLOSED STERILE ROOM

The sealed bottles of frozen plasma must be opened before placing them in the drying apparatus, and then sealed again after drying. It is most desirable, if not essential, that these procedures be carried out in a closed sterile room. When a small drier is used, it may be preferable to place the apparatus, or at least the drying chamber, in such a room. The pooling and filling of final containers should also be carried out in the sterile room when one is available.

Ideally, the closed sterile room should be large enough for the equipment and operations enumerated. The walls, ceiling, floor, windows, and doors should be impervious to the outside air and dust. The simplest way to accomplish this is to introduce cooled, dried, filtered air from outside into the room under slight pressure, so that the current through any small leaks in the room will always be from the inside out. This will also permit operation at a reasonably low temperature with dry air, desirable for ease in handling the open bottles of frozen and dried plasma. The air within the room and the external surfaces of the equipment in it can be satisfactorily sterilized by ultraviolet lights, which are ordinarily operated for about one hour before any procedure is performed. It is not necessary to operate them during the working period. However, if continuous operation is desired, protection (especially of the eyes) against burn is essential. Wearing clean gowns, caps, and surgical masks, when working in the sterile room, helps in maintaining a careful, sterile technique. Sterile gloves are unnecessary because the outside surfaces of the bottles of blood and frozen plasma are not sterile.

DRYING APPARATUS AND OPERATION

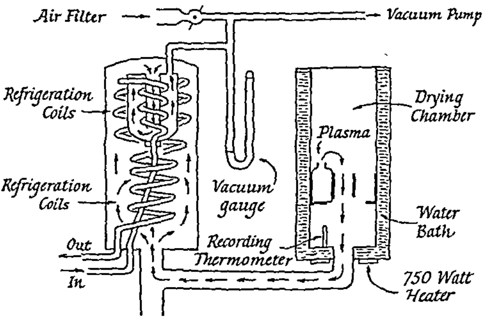
There are several varieties of apparatus for drying plasma and other biologics from the frozen state (by ice sublimation). Three will be described in some detail and others discussed briefly.

The Hot Water Jacket Drier (Strumia)

This apparatus[3,5] produces an excellent product; the mechanical controls assure uniformity of operation; it is relatively simple and economical. It incorporates the shell freezer previously described (p. 406). The unit which has been available will freeze and dry in

one day twenty-four plasma containers, each holding 300 ml. of plasma. The cost of the electrical energy for operation has been estimated by Strumia to be about $0.02 per unit of plasma. This of course does not include maintenance, technicians' salaries, other equipment for preparing plasma and for refrigerated storage, and the initial cost of the machine. At 1943 price levels, the apparatus cost about $2000.00.

APPARATUS FOR DRYING OF PLASMA



The apparatus consists of: (*a*) The shell freezer previously described (the diagram on p. 407). (*b*) A heavy cylindrical drying chamber (diagram above) surrounded by a water jacket, with the condenser outlet in the bottom. (*c*) A thermostatically controlled electric water heater, adjustable up to 80° C., fitted with a dial thermometer. (*d*) Four copper baskets with split cylindrical holders for six bottles each. Recording thermometer contacts are placed on one copper bottle holder in the lower basket. (*e*) A condenser (diagram above), with a short connection to the drying chamber. It contains refrigerated coils and baffle shields so placed as to require a long exposure to the water vapor. The vapor comes in contact with the coldest portion of the condenser last, a design which has proven adequate in operation. The condenser is cooled by a 4 cylinder, ¾ hp. compressor which operates with freon (F-12) and is equipped with a dehydrator and oil separator. It also cools the plasma sheller. A dial thermometer shows the coil temperature. An electric heater is installed in the condenser for defrosting after each drying run. (*f*) A vacuum pump, Welch Duo-Seal or Cenco-

Megavac, ½ hp., which evacuates the system to 500 microns of mercury or less within fifteen minutes with the condenser coils at −30° to −40° C. The pump is equipped with a rough dial type gauge reading from atmospheric pressure to Zero, and a Pirani or MacLeod gauge registering from 2500 microns to Zero. These components are assembled in a single cabinet with all controls, dials and gauges mounted in a central panel.

Operation

1. One hour before starting, the copper baskets from the drying chamber are placed at −20° C.

2. The containers of frozen plasma are transferred to the sterile room, which should preferably be cooled below usual room temperature. The stoppers are quickly and aseptically removed from the bottles and the necks are covered with either two layers of sterile 40 mesh gauze or sterile paper cups. The flasks are then transferred to the copper baskets.

3. The filled baskets are now placed at −20° C. for one-half hour before starting the drying operation.

4. In the meantime, the drying machine is given a test run to determine whether the required temperature and vacuum can be quickly achieved and maintained, and to cool the machine to the proper temperature for starting the run.

5. After a satisfactory test, the vacuum pump is shut off; the baskets of plasma are taken from the refrigerator, the bottle covers are removed, and the filled baskets are rapidly placed in the machine; the drying chamber is sealed tightly and the run is begun. There is some difference of opinion as to whether the necks of the bottles should be covered with two thicknesses of 40 mesh gauze, since it materially slows drying. Strumia, is among those who believe this protection against possible contamination is desirable notwithstanding,[5] while Levinson and others feel that it can safely be omitted if the technique of drying is good.[10] If a covering must be used, they prefer to elevate it over the neck of the bottle by a wire frame so that water vapor can escape freely about the sides of the protective cover.

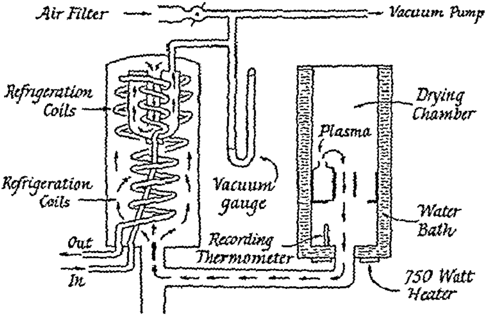
6. The vacuum must reach 500 microns of mercury or less within fifteen minutes, or the plasma will thaw.

7. After one hour of operation, the temperature of the water bath is raised from room temperature to 50° C. and maintained until the temperature of the plasma has been at this height for two hours or more. At this point drying is complete.

8. The machine is shut off and filtered air is admitted to the drying chamber through the valve mechanism and filter provided

one day twenty-four plasma containers, each holding 300 ml. of plasma. The cost of the electrical energy for operation has been estimated by Strumia to be about $0.02 per unit of plasma. This of course does not include maintenance, technicians' salaries, other equipment for preparing plasma and for refrigerated storage, and the initial cost of the machine. At 1943 price levels, the apparatus cost about $2000.00.

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and the second to $-40°$ C. A plate type of condenser forms the top of the drying chamber and is less than 10 cm. from the top of the plasma bottles. The drying chamber is designed with the exhaust to the vacuum pump located in the bottom. This results in a small amount of dead space not requiring a large pump capacity (a Hypervac 20 pump is satisfactory). The bottle holders consist of guide frames, in which the bottles fit, surrounded by reflectors. Inside of the reflectors are coils of Nichrome V wire as the source of infra-red heat. This entire assembly is surrounded by a polished aluminum cylinder, painted black on the outside to reduce the heat loss. Uniform resistance of each heating unit is carefully adjusted to assure uniform drying. The drier is compactly assembled with outside controls, including a thermocouple-vacuum control which will stop the heating of the plasma if the vacuum exceeds 100 microns. Indicators show the vacuum and the temperature of the condenser and the plasma. The application of heat during the drying cycle can be made automatic by the use of a properly cut motor-operated templet to actuate a thermocouple which controls the Variac transformer governing the electric current supply to the heating units. The control relays may also be actuated by time clocks. After a run, the ice (very brittle because it contains no air) is relatively easily chipped off the condenser plate, and the machine can be made ready and a second run started within one to one and one-half hours.

Operation

1. Before actual operation, the machine is started to bring the cold plate condenser to $-40°$ C., to cool the drying chamber and bottle holders and to test the vacuum.

2. The containers of frozen plasma are handled as described in steps 2 and 5 on page 411, except that the bottle holders are cooled in the machine.

3. As soon as the machine is ready and the prepared bottles of plasma placed inside (a thermocouple is inserted in one bottle to record the plasma temperature), the drying chamber is sealed tight and the run is begun.

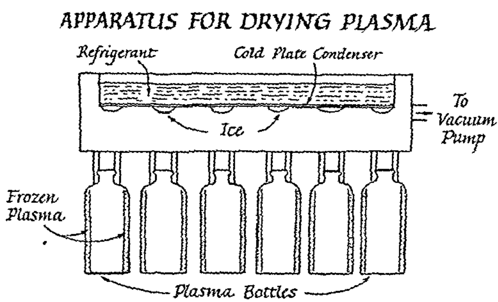
4. The vacuum in this apparatus will reach 100 microns of mercury in ten minutes and, when this is achieved, the application of heat is begun. The vacuum should never exceed 100 microns at any time during the drying cycle and is as low as 20 microns in the terminal drying period.

5. The drying cycle occupies nine hours when the plasma has been bottled in 300 ml. amounts. The standard heat requirements for this cycle are successively: 100 watts for two hours, 60 watts for

for this purpose. The entering air is dried by passing through the condenser into the drying chamber. Dry filtered nitrogen may also be used to release the vacuum and must be employed if the plasma bottle is not to be evacuated for storage.

9. The bottles are again covered, if the covering was removed for drying. They are removed from the drying chamber and quickly stoppered in the sterile room. The bottles must now be evacuated (p. 384), unless a mechanical vacuum-stoppering machine was employed, so that the plasma is stored in an inert environment. Even if the vacuum was released with nitrogen, evacuation of the bottle is desirable to facilitate reconstitution of the plasma.

10. The drying cycle under the above conditions requires about eighteen hours to produce dried plasma with less than 1 per cent moisture.



The Infra-Red Drier (Levinson and Oppenheimer)

This apparatus[9,10] employs an ideal type of heating for the purpose (p. 403). It has a very short drying cycle and easily operated controls. The installation is quite compact. The cost of apparatus, operation, and maintenance is considerably greater than the Strumia equipment. The mechanical controls assure a uniform product and the operation is relatively simple. The machine dries the plasma in half the time taken by the Strumia apparatus and produces an excellent product. The shell freezer must be procured and operated independently of the drier. In nine hours the unit will dry twenty-eight plasma containers, each holding 300 ml. of plasma. The apparatus (diagram above) has a refrigerating system of the cascade type, embodying two stages of cooling, the first to −25° C.

and the second to $-40°$ C. A plate type of condenser forms the top of the drying chamber and is less than 10 cm. from the top of the plasma bottles. The drying chamber is designed with the exhaust to the vacuum pump located in the bottom. This results in a small amount of dead space not requiring a large pump capacity (a Hypervac 20 pump is satisfactory). The bottle holders consist of guide frames, in which the bottles fit, surrounded by reflectors. Inside of the reflectors are coils of Nichrome V wire as the source of infra-red heat. This entire assembly is surrounded by a polished aluminum cylinder, painted black on the outside to reduce the heat loss. Uniform resistance of each heating unit is carefully adjusted to assure uniform drying. The drier is compactly assembled with outside controls, including a thermocouple-vacuum control which will stop the heating of the plasma if the vacuum exceeds 100 microns. Indicators show the vacuum and the temperature of the condenser and the plasma. The application of heat during the drying cycle can be made automatic by the use of a properly cut motor-operated templet to actuate a thermocouple which controls the Variac transformer governing the electric current supply to the heating units. The control relays may also be actuated by time clocks. After a run, the ice (very brittle because it contains no air) is relatively easily chipped off the condenser plate, and the machine can be made ready and a second run started within one to one and one-half hours.

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3. As soon as the machine is ready and the prepared bottles of plasma placed inside (a thermocouple is inserted in one bottle to record the plasma temperature), the drying chamber is sealed tight and the run is begun.

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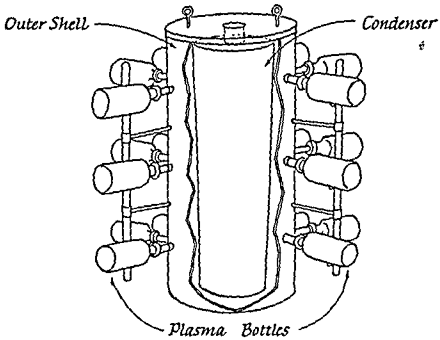
one hour, 40 watts for one hour, 20 watts for one hour, 12 watts for one hour, and 9 watts for three hours. The terminal heating is slight to avoid the possibility of overheating the plasma in bottles which may have dried more rapidly than some of the others.

6. On completion of the cycle, the machine is turned off and the vacuum released as described in step 8, page 411.

7. The bottles of dried plasma are removed and stoppered as described in step 9, page 412.

8. The product obtained in the nine hour cycle is excellent; the dried plasma has a relatively large volume, dissolves almost instantaneously and contains *less than 0.1 per cent* moisture.

APPARATUS FOR DRYING PLASMA



The Warm Air ("Pig") Drier (Wyckoff)

This apparatus[1] produces a satisfactory product. It is inexpensive, costing only about $300.00 in 1943. It is portable and simple to operate. Dry ice and alcohol are used to refrigerate the condenser. The shell freezer must be procured and operated as a separate unit.

The apparatus (diagram above) consists of an outer cylindrical shell, the *drying chamber*, with an evenly spaced series of thirty tubes to which the bottles of plasma are directly attached for drying; a coaxial inner cylinder, the *curved surface condenser*, attached to the cover, which seals the outer cylinder and which has a central open-

ing for filling the condenser with dry ice and alcohol. The drying chamber is attached to either a Cenco-Megavac or Welch Duo-seal vacuum pump. The diameter of the thirty tubes on the drying chamber should be the same as the diameter of the neck of the plasma bottle. The connection is made by a short length of larger but tightly fitting heavy rubber tubing. Any tubes not in use are closed with greased rubber stoppers. A MacLeod gauge is attached to the vacuum line, close to the drying chamber. A series of several such driers has been successfully operated as a unit, when larger capacity is desired.

OPERATION

1. Before actual operation, the machine is started with all drying tubes stoppered to test the vacuum. The condenser is charged with dry ice and alcohol to bring it to the proper temperature for the run.
2. The containers of frozen plasma are handled as has been previously described (step 2 and 5, p. 411) except that no bottle holders are used. Although a protective covering should be used in transfer, the neck of the bottle cannot be covered in this apparatus.
3. The proper number of rubber connections are fixed to the drying tubes in such a way as to provide an even distribution of the plasma bottles. The prepared bottles are brought from the cold room and rapidly attached to the tubes. The short heavy connecting tubes will ordinarily provide sufficient support for the bottles, although a wire frame may be used for additional support if desired.
4. The vacuum pump is started at once when all bottles have been attached. The drying may be carried out at room temperature or, as soon as the vacuum reaches 100 microns of mercury or less, the room air may be heated to a temperature of 43° C. to reduce the time of drying (p. 403). A vacuum of 500 microns or less must be obtained within fifteen minutes or the plasma will thaw. Dry ice is added to the condenser from time to time as necessary.
5. The drying cycle at 43° C. is approximately sixteen hours when the plasma is in 300 ml. lots. Completion of the drying is judged by observation of the plasma bottles. Experience will quickly permit accurate judgment.
6. The vacuum is released by filtered air or dry, filtered nitrogen introduced through a "T" in the vacuum line (step 8, p. 411).
7. The bottles of dried plasma are removed and stoppered as described in step 9, page 412.
8. The product obtained in the sixteen hour cycle is of good quality and can be brought to a residual moisture content of 0.2 per cent.

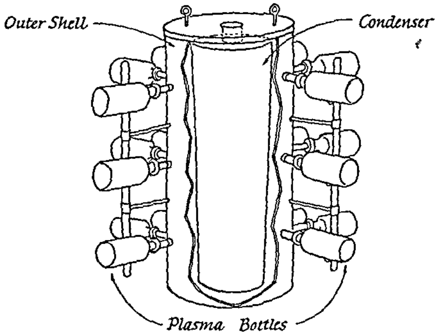
one hour, 40 watts for one hour, 20 watts for one hour, 12 watts for one hour, and 9 watts for three hours. The terminal heating is slight to avoid the possibility of overheating the plasma in bottles which may have dried more rapidly than some of the others.

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Other Types of Drying Apparatus

There are several other types of apparatus which have been used for drying blood plasma.[2,4,7,8,9] The three described represent the most satisfactory now available. It is possible that a machine employing electronic energy for heating will prove to be better than other types, but it will need to be much less expensive than it would be at the present time to be practical. Two methods which have been advocated deserve comment.

The Desivac machine, developed by Flosdorf and Mudd, employs hot water heat and a chemical desiccant.[2] The disadvantages of this method of drying have been discussed (p. 402).

The Adtevac process, described by Hill,[4] also employs a chemical desiccant. This process, as described in the literature, has some serious faults in the opinion of the authors. Although mechanically efficient and saving of time, the practice of pooling whole blood of different groups and separating the plasma by a De Laval centrifuge results in the presence of a considerable amount of cell debris and free hemoglobin in the plasma. While the reported results would indicate that the amounts ordinarily found in such plasma are not clinically dangerous their presence is undesirable. Snap freezing by rapid evacuation of containers of liquid plasma does not produce a shell which is desirable for satisfactory drying, but tends to spatter plasma in small droplets which dry without freezing and become relatively insoluble. Finally, the plasma must be scraped from the container in which it was dried, mechanically pulverized, and transferred to another container for storage and use; all these procedures are violations of the closed system.

PACKAGING AND STORAGE OF DRIED PLASMA

Since a prominent advantage of dried plasma is its ready transportability and wide range of permissible storage temperatures, it should ordinarily be packaged with a bottle of sterile 0.1 per cent citric acid in distilled water (of a volume equivalent to the original plasma), a double-ended needle for transfer of the fluid, and a sterile filter set for administration. For hospital use such packaging, although desirable, is not necessary.

Blood plasma, properly dried and sealed, containing less than 1 per cent residual moisture may be safely and satisfactorily stored for at least five years. Storage should preferably be in a dry, cool place (20° to 37° C.), although the plasma will not be harmed by extreme cold or temperatures up to 80° C., if the moisture content is less than 0.5 per cent. Temperatures below 0° C. will freeze the diluent with possible breakage of its container. If the bottle is un-

broken, the diluent may be used after thawing. If the temperature of the diluent is much above 37° C. at the time of reconstitution, the plasma proteins undergo varying degrees of denaturation, or even coagulation, and the plasma is unsafe for use. The standard five-year dating period is based primarily on the anticipated ability of the rubber stopper to maintain an airtight seal. Theoretically, dried plasma may be safely stored almost indefinitely at ordinary temperatures without deterioration.

ADMINISTRATION OF DRIED PLASMA

Dried plasma is reconstituted for administration by the addition of the desired amount of sterile, pyrogen-free 0.1 per cent citric acid in distilled water. Ordinarily, the preferred amount is that which will restore the plasma to its original volume. If the blood was collected in a preservative solution containing sufficient citric acid, for example an ACD solution, sterile distilled water is used without the added citric acid. The temperature of the diluent at the time of reconstitution should be above 0° C. and below 37° C. (see previous paragraph). If the bottle of dried plasma has been evacuated, the diluent can be transferred without opening the bottles. After cleansing the tops of the stoppers of the plasma and diluent bottles, a double-ended needle is inserted into the bottle of diluent, which is then inverted and the other end of the needle placed through the plasma stopper, allowing the vacuum to draw the diluent into the plasma. If no vacuum is present, it is permissible to open the bottles and pour in the diluent, since dried plasma should *always be administered at once after reconstitution* to avoid the danger of growth of a chance contaminant. If more than one hour elapses before administration, the plasma should be discarded. Properly dried and sealed plasma will go into solution completely within two to three minutes. The resulting liquid is always slightly cloudy. If it does not go into solution promptly, it is likely that some denaturation of the protein has occurred and it *should not be used.*

Reconstituted dried plasma is administered to the patient in the same manner as plasma which has been stored in the liquid state (p. 389).

SELECTED EXCERPTS FROM "MINIMUM REQUIREMENTS"*

Method of Handling Plasma or Serum to be Dried. When plasma or serum is to be processed to the dried state, shell freezing shall follow

* From "Minimum Re[illegible]
mal Human Serum [illegible]
ments are mandato[illegible]
commerce.

filling without needless delay and the drying is preferably begun without delay after shell freezing. However, the shell-frozen plasma or serum may be stored at a sufficiently low temperature (preferably −18° C. or lower) to satisfactorily hold it in the frozen state, to await the outcome of the sterility test or to await a more convenient time for carrying out the drying process. In either event, the final disposition of the entire lot shall depend upon the results of the tests for sterility. . . . Drying shall be accomplished by a method which is not deleterious to the plasma or serum constituents and which will result in a readily soluble and sterile product. In general, the finished dried product is of a finer quality if the drying is completed relatively soon after being brought to the frozen state.

In the case of dried plasma or serum the label or an accompanying circular shall give full directions for re-dissolving the dried plasma or serum as applicable to the particular type of container used and full directions for getting the restored plasma or serum from the container into the recipient in an aseptic manner.

The Diluent. It is recommended that a suitable glass container (U.S.P. Type I glass) holding the necessary amount of pyrogen-free, sterile, and otherwise suitable diluent shall accompany each container of dried plasma or serum. This container shall be closed after filling with an air, moisture and bacteria impermeable seal. The quantity of diluent in the container shall be enough to restore the plasma or serum to its original volume. The container shall be equipped so that the necessary connections for the transfer of the diluent to the dried plasma or serum may be attached easily, directly, and aseptically.

However, the above requirement shall not be effective if specific requests for the dried plasma or serum without the diluent are made by hospitals, clinics, etc. When dried plasma or serum is dispensed in this manner, the accompanying circular shall give, in addition to other directions, explicit directions and warnings as to the kind of diluent required, the quantity to be used, and any other essential information in order to safeguard the recipient.

When dried plasma has been processed from whole blood containing sodium citrate as the anticoagulant the diluent recommended is 0.1 per cent solution of citric acid in pyrogen-free sterile distilled water. When the anticoagulant is ACD solution the diluent recommended is the same water without citric acid. The latter also is recommended as the diluent for dried serum.

Dried Plasma or Serum. The expiration date for dried plasma or serum shall not exceed five years from the date of manufacture, provided the moisture at all times is less than 1.00 per cent as determined by the (acceptable) method. . . . (see below). The date of manufacture is calculated as the date of bleeding the donor.

Dried plasma or serum may be stored at the prevailing temperature provided this does not exceed 37° C (98.6° F.). It is recognized that brief exposures to high temperatures are not particularly damaging to dried plasma or serum, nevertheless this is not acceptable as a continued practice. Dried plasma or serum which is intended for use because of its anti-

body content should be stored at 2° to 10° C. as recommended for immune serums.

A Method for the Determination of Residual Moisture

The amount of residual moisture remaining in a product which is labeled as dried shall contain not more than 1.00 per cent moisture when determined by the following method: "Expose a 1 to 2 gm. sample of the product, accurately weighed to the third decimal, evenly distributed in a weighing bottle not less than 60 mm. in diameter, in a vacuum desiccator at less than 1 mm. pressure, over fresh phosphorus pentoxide, and at room temperature until the weight remains constant to the third decimal." In removing the sample to the weighing bottle, it is important to avoid unnecessary exposure of the dried product to the air, particularly if the moisture content of the atmosphere is relatively high. Samples of less weight may be used in smaller weighing bottles for the determination of moisture in products where full samples are not available.

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CHAPTER 19

Convalescent and Hyperimmune Plasma and Serum

By John B. Alsever

The development of the present use of normal, convalescent and hyperimmune plasma and serum in the prophylaxis and treatment of acute infectious diseases is due largely to the work which has been done by the serum centers located in several of the larger cities, notably New York, Chicago, Philadelphia, and Los Angeles.[5,8,9,10] These were founded and operated principally because of the keen interest of a few men in the possibilities in this field of therapy. These laboratories have employed serum rather than plasma, since serum has always been the standard form of biologic employed for immune therapy and plasma has not been used clinically to any extent until recent years. Serum possesses certain advantages. It can be filtered for sterility and clarity and stored at 4° to 10° C., as is customary for biologics, since the fibrinogen is removed in its preparation. However, in view of the widespread establishment of blood banks and facilities for storing plasma in the frozen state or drying it, it is logical to expect that the serum centers of the past will become a part of the general blood transfusion service in hospitals and communities and that plasma rather than serum will be prepared for use in immune therapy. It is possible that the employment of plasma and serum for this purpose will eventually be considerably less if programs of plasma fractionation (Chaps. 2 and 22) become sufficiently developed to provide economically adequate quantities of the concentrated (immune) gamma globulin fraction for general use in the future. The globulin preparation is considered preferable for immune therapy in measles and it is effective in some other diseases (Chap. 22).

The usefulness of human plasma, either normal, convalescent, or hyperimmune, in the prophylaxis and treatment of measles,

whooping cough, scarlet fever and mumps has been well established. There is evidence that it may be extremely useful in several other diseases. This aspect of adequate blood plasma transfusion services is definitely within the field of public health and has stimulated several state and local health departments, during the past three or four years, to take an active part in the development of such programs (Chap. 25). The great advantage of employing human rather than animal plasma or serum for immune therapy lies in the fact that the administration of a heterologous protein, with such attendant dangers as anaphylaxis and serum sickness, is avoided.

PREPARATION OF CONVALESCENT PLASMA

Convalescent plasma may be prepared by any of the methods described in the three previous chapters, since the antibody content deteriorates quite slowly in liquid plasma stored at room temperature, apparently remaining at good strength for about four to six months, and is indefinitely stable in frozen and dried plasma. It would seem preferable, however, to prepare convalescent plasma as fresh plasma and either dry it or store it in the frozen state to insure full content of antibodies, prothrombin, and complement. The latter may be particularly important in certain cases (p. 426). The preparation of frozen or dried plasma is also preferable since the use of concentrated plasma is advantageous. The only real difference, then, between the preparation of normal and convalescent plasma lies in the selection of the donors.

Convalescent plasma is prepared by selecting and bleeding donors within four to six months after recovery from the specific disease for which immune plasma is desired. At this time the antibody content of the donor's blood should still be at a high level and the physical condition of the donor should permit the withdrawal of the usual amount of blood (300 to 500 ml.) without adversely affecting his state of health. Convalescent plasma has been found generally to be about four times as potent as pooled normal adult plasma.

PREPARATION OF HYPERIMMUNE PLASMA

The Philadelphia Serum Center has developed the preparation and use of hyperimmune serum* in the prophylaxis and treatment of whooping cough with excellent results.[9,22] Although this

* Serum is prepared by the Philadelphia Serum Center but plasma may be used equally well.

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The usefulness of human plasma, either normal, convalescent, or hyperimmune, in the prophylaxis and treatment of measles,

administration of ten times the prophylactic dose of convalescent plasma is of value in modifying the disease in the Koplik spot stage and may be of some value in lessening the acute manifestations of the disease after the eruption has appeared.

Gamma globulin and, therefore, plasma as well, may be of value in the prophylaxis of German measles (rubella) which is of importance during pregnancy (p. 450).

Scarlet Fever

Convalescent scarlet fever plasma and serum have proven strikingly effective in preventing the disease after exposure. When used as treatment after acute illness has begun, they may produce complete remission or marked lessening of the toxic manifestations in twelve to twenty-four hours.[illegible] Pooled normal adult plasma is also effective if given in quadrupled dosage. For prophylaxis, 10 to 40 ml. of convalescent plasma, depending on age, given intramuscularly within twenty-four to forty-eight hours after exposure, will reduce the incidence from the expected 10 to 15 per cent to 1 per cent. Since the duration of this passive immunity is from ten to fourteen days, repetition of the protective dose every ten days is required if exposure continues.

In the treatment of scarlet fever, the convalescent plasma or serum is given intravenously in doses of 20 to 100 ml., depending on the age of the patient. Treatment should be begun within thirty-six hours after onset and the dose repeated every forty-eight hours until the acute manifestations have subsided. Improvement occurs within twelve to twenty-four hours after injection in 75 to 85 per cent of cases. The incidence of complications has been found to be about 50 per cent less in the treated group and the sequelae which occur are milder. However, notable benefit may be expected from the use of convalescent plasma up to the sixth day of the disease in uncomplicated cases. In the presence of complications, considerable benefit may be noted if at least three injections of the amounts indicated previously are given at eight hour intervals.

It should be noted that plasma, with a high content of antibodies against the streptococcus of scarlet fever, will give good results in many other hemolytic streptococcus infections, such as erysipelas, pharyngitis, cervical adenitis, postoperative complications of mastoiditis, and pneumonia. Experience has indicated that there is at times a synergistic effect of combining the use of convalescent plasma and the sulfonamides. This should be of particular value in the treatment of serious or difficult cases.

method has not been employed as yet in other diseases, its possibilities should be investigated. The procedure which is employed in preparing the serum is as follows. Donors are selected who are known to have had whooping cough, who are 21 to 30 years old, and who are willing to receive a course of immunizing injections of pertussis vaccine. Three courses are given at four month intervals, each course consisting of three weekly injections of 2 ml., 3 ml. and 3 ml., respectively, of Sauer vaccine, containing 10 billion organisms per ml. The donors are bled one month after completion of the third course of injections. The agglutinin titer of the hyperimmune serum reaches 1/1280 to 1/5120, as compared with a titer of 1/300 in the average convalescent donor. Hyperimmune plasma or serum is prepared from the bleedings and stored in the same manner as convalescent plasma.

PLASMA IN PROPHYLAXIS AND TREATMENT OF INFECTIOUS DISEASES

Measles

Both convalescent and pooled normal adult plasma and serum have been used satisfactorily in the prophylaxis of measles (rubeola) and in the treatment of the disease during the preeruptive stage.[5,6,7,8,9] Prevention of the disease after exposure is particularly important in (*a*) children under four years of age, (*b*) susceptible persons who are debilitated because of another illness or injury, and (*c*) pregnant women, since the mortality is highest and the complications are most severe in these groups. The usual dose intramuscularly of convalescent plasma for the exposed child is 10 to 20 ml., depending on age (four times this amount if normal plasma is used), within seven days after exposure. Complete protection is obtained in 50 to 70 per cent and modification in 20 to 40 per cent. Since such passive immunity lasts only ten to fourteen days, the injection should be repeated if exposure continues. In children over five years it is desirable to attempt to modify the disease rather than prevent it, as a mild illness is believed to confer as good and as lasting an immunity as the unmodified illness. This can ordinarily be done by giving about half the dose required for complete protection. Larger doses, up to 50 to 75 ml., are required to protect adults. Concentrated gamma globulin has proven preferable for the prophylaxis of measles. At the present time it is available from the war surplus being distributed by the American National Red Cross through state health departments (p. 449).

There is also some evidence which indicates that the intravenous

administration of ten times the prophylactic dose of convalescent plasma is of value in modifying the disease in the Koplik spot stage and may be of some value in lessening the acute manifestations of the disease after the eruption has appeared.

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of the acute disease and lessening the amount of paralysis. He has employed the administration of 60 ml., plus one ml. per pound, (0.45 kilogram) of body weight, repeated every twelve to twenty-four hours, ordinarily for one to three doses. He has also indicated that twice the dosage of pooled normal adult plasma or serum gave equally as good results. However, the carefully controlled studies carried out during the summer of 1944 by the New York State Department of Health on acute cases in patients from one to twelve years old, using large doses of concentrated gamma globulin (equivalent to 500 to 2500 ml. of normal plasma), indicated that immune therapy is without any detectable benefit in the acute disease (p. 450).

The dose of gamma globulin employed in the New York State study exceeded the total amounts of plasma or serum recommended and employed by Levinson and was given in one injection. Therefore, if immune therapy is actually effective, the New York study should have produced more clear-cut results than those observed with the use of divided doses of normal or convalescent plasma or serum. It seems possible that the apparent benefit observed by Levinson may have been due to the bolstering of the weakened resistance of these patients by the addition of nonspecific antibodies and complement (p. 426).

While it is possible that plasma or serum, either convalescent or normal, might be effective as a prophylactic measure, such use is quite impractical, even experimentally, since half of a rather large population should be given protective doses every ten to fourteen days during the entire season to determine its effectiveness. If used as a general measure to control outbreaks of the disease, injections should be given similarly to all persons in the area who had not acquired immunity by contracting the disease previously.

Other Acute Infections

It is likely that immune therapy may be of real value in several other infectious diseases. Its trial in a few has already been reported.

Virus Pneumonia. This has been successfully treated by both convalescent and pooled normal adult plasma or serum.[5,16,17] The administration of doses of 100 to 500 ml. resulted in a prompt fall in temperature, recovery from the acute symptoms and shortening of the period of convalescence in the majority of cases treated.

Epidemic Keratoconjunctivitis. Striking improvement of the disease has been observed with the use of convalescent plasma or serum in a small series of cases.[18]

Infectious Hepatitis. Since gamma globulin has proven an

Mumps

Convalescent mumps plasma or serum is reported to be 90 to 95 per cent effective in preventing the disease in exposed individuals.[2,3,4,5,6,8,9] The dose required is 20 to 40 ml., depending on the age of the patient, given intramuscularly within one week of exposure. Such a procedure is important chiefly in avoiding the danger of orchitis in adolescents and adults. Thalhimer and others report that a dose of 40 ml. intravenously will cause prompt subsidence of acute orchitis in most instances. Normal pooled adult plasma was used considerably in the Navy during World War II and found to be rather dramatically effective in acute orchitis when given in doses of 500 ml. at the time orchitis appeared. Doses of 100 ml. as a prophylactic measure at the onset of mumps did not appear to decrease the incidence of orchitis.

Whooping Cough

Excellent results have been obtained in the prophylaxis and treatment of whooping cough with the use of hyperimmune plasma or serum,[9,22] the preparation of which has been previously described. The protective dose is 10 to 20 ml., depending on the age of the patient, given intramuscularly during the first week after exposure. In a series of 215 infants and young children studied by McGuinness and his coworkers complete protection occurred in 78 per cent of the cases, 10 per cent had a very mild illness, 6 per cent moderate, and only 6 per cent had typical pertussis, but without complications. They recommend that the protective dose be repeated every five to seven days if exposure continues.

The hyperimmune material is also effective in treatment of the acute disease. McGuinness, reporting on the results in 315 cases of which 123 were infants of six months or younger, found that intramuscular administration of 20 ml. daily for three or four days resulted in subsidence of the major symptoms within ten to fourteen days in 69 per cent of the cases, and a death rate of only 1.5 per cent. He recommends that larger doses, 60 to 100 ml., should be injected intravenously, particularly in critically ill infants, and repeated if necessary.

Anterior Poliomyelitis

This disease is admittedly a most difficult one in which to judge the effectiveness of any therapy.[1,5,9,14,15,21] Levinson, in particular, has long advocated the use of convalescent poliomyelitis plasma or serum in the treatment of the acute disease and has been convinced that its use has been of definite value in causing a remission

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effective prophylactic agent in the epidemic form of this disease (p. 450), convalescent and normal pooled adult plasma or serum *should also be effective in proper dosage if one can be assured that* it is noninfectious (p. 357).

Pneumococcus Pneumonia. A most interesting type of response to plasma therapy occurs in this disease. Taplin,[19] while studying the value of specific antisera in the treatment of the disease, observed that a crisis failed to occur in several cases, although there was an excess of antibody in the patient's blood as determined by the Francis test. In searching for a possible reason, it was found that these cases showed a markedly deficient *blood complement.* The results of the administration of complement in the form of properly prepared plasma were indeed striking; a typical crisis occurred within twenty-four hours.

Deficiency of Blood Complement

The occurrence of a low content of complement in the blood in acute illnesses was investigated in 1941 by a group at the Syracuse University College of Medicine[5,20] who had become interested in Taplin's observations regarding the importance of lack of blood complement in certain cases of severe pneumococcus pneumonia. It was found, in addition, that blood complement was at times low or practically absent in several other acute infections and acute manifestations of allergy. There was opportunity to study the effect of therapy in only two cases. A deficient blood complement was found on the first day of symptoms in a case of serum sickness and on the first day of abnormal urinary findings in a case of acute nephritis complicating scarlet fever. Striking clinical improvement was observed in both cases following the administration of 300 ml. of fresh plasma. Further study is indicated along these lines to prove or disprove these initial observations and to explore the effect of the administration of complement in other conditions in which it may be found to be low.

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by the closed system, using commercial vacuum containers. In a later article[6] the same authors compared an extended series of 3,384 such transfusions with 1500 reports on transfusions of commercially prepared dried plasma. The former series showed a significantly smaller reaction rate. The experience of others,[12] including the authors, has shown that a rate below 1 per cent is possible with plasma in a good hospital transfusion service. The goal is more easily accomplished if expendable tubing is employed for the administration sets.

PYROGENIC REACTIONS

Contamination by pyrogenic substances is responsible for the great majority of untoward reactions associated with the use of plasma. These complications are largely preventable and in any well-conducted transfusion service should occur quite infrequently.

Clinical Description. During the plasma transfusion, or soon after, the recipient develops a chill of varying severity. This is typically followed by a temperature rise of 1° to 4° F. and persists for a varying period of time up to five or six hours. Sometimes the fever is observed without a definite chill and occasionally a chill without any subsequent elevation of temperature (Chap. 12).

Miller and Tisdall,[4] in a study of 10,000 administrations of pooled plasma, reported 2 per cent pyrogenic reactions. Most of these were mild, developed within one hour after beginning the injection, and showed an average temperature rise to 103° F. The time of onset varied from immediately to twelve hours, the duration from three minutes to twelve hours, and the temperature rise from 99.4° F. to 111° F. The immediate source of the pyrogenic material was reported to be about equally divided between the plasma itself and the administration sets, with the latter being implicated slightly more often

Treatment. If the reaction is severe, the transfusion should be discontinued, otherwise no other than symptomatic treatment is necessary. Consult Chapter 12.

Sources of Pyrogens. In the preparation and administration of blood plasma or serum there are several possible sources of contamination by pyrogenic material (Chap. 27): (1) Contamination of the transfusion equipment and the solutions or distilled-water by nonpathogenic water-borne bacteria. (2) Contamination of plasma by pathogenic and nonpathogenic bacteria. It is possible, for reasons which have been discussed in Chapters 17 and 18, that small numbers of bacteria may exist undetected in frozen or dried plasma. However, bacterial contamination is only remotely

CHAPTER 20

Complications from Plasma Infusion

By John B. Alsever

Although there are a relatively large number of possible causes of untoward reactions resulting from the administration of plasma or serum, the use of a product carefully prepared and administered by acceptable methods greatly minimizes their occurrence and should result in a reaction rate of 1 per cent or less. The complications from the injection of plasma are essentially the same as those seen with whole blood (Chap. 12) except for the rarity of hemolytic reactions.

INCIDENCE OF REACTIONS TO PLASMA

Reactions to the transfusion of plasma are considerably less frequent than with the use of whole blood. Reports of the reactions observed in studies of large numbers of plasma transfusions, some of which have been mentioned previously, indicate that the reaction rate can be brought to 1 per cent or less. Miller and Tisdall,[4] in a series of 10,000 liquid plasma transfusions, reported a reaction rate of 2.96 per cent, of which 2 per cent were thermal and presumably preventable. The study was conducted during the war in Army hospitals where the administration sets were prepared in the installations but the plasma was prepared in an Army center. In both instances the frequent turnover of technical personnel was largely responsible for the high reaction rate. Weinstein[7] reported a reaction rate of 1 per cent in 1500 transfusions of unpooled plasma. Lozner and Newhouser[17] recorded 1.1 per cent reactions in a series of 1751 transfusions of liquid plasma prepared

to which the patient is sensitive is inadvertently introduced. Dickstein[18] reported a case of the latter type where severe generalized urticaria, cyanosis and cessation of respiration attended a plasma transfusion. Maunsell's[11] observations illustrate the frequency of reactions in sensitive individuals with hereditary types of allergy. Seventy-seven per cent of such persons showed positive skin tests to various lots of plasma as compared to only 20 per cent in the nonallergic control group, and in those transfused, there were allergic reactions in fourteen out of seventeen allergic patients and none in twenty-one nonallergic controls. Maunsell also observed that repeated transfusion in both groups not only did not lead to the development of sensitization but in the allergic group desensitization was observed in ten of the eleven cases so studied.

Treatment. The allergic reactions are temporary in nature, mild to moderate in intensity as a rule, and do not necessitate discontinuing the transfusion. However, the appearance of a severe allergic manifestation is an indication for prompt discontinuance of the infusion and may very rarely require heroic measures. Epinephrine hydrochloride is the drug of choice for relief of the acute symptoms. The 1/1000 solution should be given subcutaneously or intramuscularly in doses of 0.3 to 0.5 ml.

Prevention of Allergic Reactions. Complete avoidance of allergic reactions is quite impossible. However, the incidence of reactions due to passive transfer may be reduced by rejecting as blood donors persons with active allergic manifestations. Reactions in sensitive persons may be minimized by the use of fasting donors. The blood of fasting donors might contain and transmit pollens if the blood was collected during or immediately after the donor had inhaled them. Therefore, in the case of patients very sensitive to pollens, dusts, or danders, who require transfusion, special precautions possibly should be taken, if the circumstances permit, in regard to the selection of donors, and the plasma then prepared specifically for this patient.

TRANSMISSION OF INFECTIONS BY PLASMA

The transmission of syphilis, malaria, and homologous serum jaundice has been discussed on pages 234, 293, and 355, respectively. In addition, it is possible to transmit other infections by the use of plasma contaminated with the specific pathogenic organism. In such a case, the organism is either present in the donor's blood, and capable of growing and surviving during plasma preparation, or is introduced by accidental contamination during the processing of the plasma. Neither accident should occur in a well-run trans-

possible in liquid plasma unless it is used before the completion of the sterility tests on the final containers. Miller and Tisdall[4] reported a 1 per cent incidence of bacterial contamination in their wartime experience with the preparation of over 70,000 units of liquid plasma using the commercial vacuum type containers. This rate is relatively high and is largely explained by the rather rapid turnover of technical personnel. In a well-run laboratory where the workers are both well trained and relatively permanent, this type of contamination should almost never occur. (3) Impure chemicals. (4) Denatured plasma proteins. Improper heating of blood plasma at the time of administration or during the thawing of frozen plasma, as well as improper drying of blood plasma due to excessive application of heat, causes varying degrees of denaturation. In addition to being potentially toxic, these changed human proteins may cause a typical thermal reaction.

Prevention of Contamination by Pyrogens. Thermal reactions may be largely prevented by the proper preparation of the equipment and fluids employed in the transfusion service (Chap. 27), avoidance of accidental contamination during the collection of blood and the processing of plasma, the use of pure, pyrogen-free chemicals, and the proper application of heat in the thawing or drying of frozen plasma and in the administration of plasma.

ALLERGIC REACTIONS

Various types of allergic reactions are not infrequently observed in the administration of blood plasma.

Clinical Description. Allergic reactions are most often urticarial. When severe, they may take the form of angioneurotic edema or an anaphylactic type of reaction. These and other types which have been observed apparently are due either to the passive transfer of antibodies, or to the inadvertent introduction of a protein to which the patient is already sensitive. Instances of hay fever[9] and asthma[4] have been observed.

In the series of 10,000 administrations of plasma reported by Miller and Tisdall[4] there were a total of 1.05 per cent of allergic reactions. The time of onset varied from immediate to seven and one-half hours. Urticaria was observed in 102 patients, about one half local and one half generalized; three were asthmatic. The 105 reactions occurred in eighty-one patients, six had a total of twenty-four reactions; about 76 per cent were mild, 20 per cent moderate, and 4 per cent severe. Allergic reactions are more common in patients already sensitive to one or more proteins, and the likelihood of severe reactions is enhanced if one of the proteins

of plasma reactions to sensitivity to the A and B factors present in the plasma. They contended that these factors are toxic and that the reactions can be predicted by skin tests, which they found to be positive in 20 per cent of the persons tested. Their work has not been confirmed and Maunsell's study shows their findings can be explained on an allergic basis (see *Allergic Reactions*). Maunsell's observations[12] demonstrated that there was no relation between group-specific substances and positive skin tests.

Prevention. The use of Witebsky's A and B specific substances to neutralize the plasma agglutinins may be employed where any question exists as to the safety of plasma for transfusion, whether pooled or unpooled.

TOXIC REACTIONS

Among the possible causes of toxic reactions, which are the result of damage to vital organs, are:

Red Cell Stromata. Red cells may be aspirated with the plasma if centrifugation is not complete and will subsequently hemolyze, leaving stromata. Separation of the plasma by the De Laval type of centrifuge, where pooling of the whole blood precedes centrifugation, is particularly prone to produce red cell contamination of plasma. The clinical significance of red cell stroma is discussed on page 274.

Denatured Plasma Proteins. These are quite toxic and may cause fatal damage to the organism if injected in large quantity as in the case of overheated plasma. Overheating is likely to interfere seriously with administration by coagulation or by the formation of insoluble particles of protein.

Reactions with Fresh Serum. A high reaction rate has been reported from the administration of freshly prepared serum and was generally attributed to a toxic substance liberated by the conversion of fibrinogen to fibrin in the clotting of the blood. This theory originated before pyrogens were known and it is quite likely, although there is not complete proof, that these reactions were almost entirely due to pyrogen contamination.

Toxic Chemicals. The mercury compounds, which formerly were routinely added as bacteriostatic agents (p. 387) in the preparation of plasma, are potentially toxic because of their mercury content. These were either merthiolate, in a 1/10,000 final concentration, or phenyl mercuric borate or nitrate, in a 1/15,000 concentration. There is a theoretical risk of renal damage in the administration within twenty-four hours of more than two to three liters of plasma with such amounts of mercury to patients

fusion service. The former will be avoided by proper selection of the donor, the latter by the use of proper technique in processing the plasma.

REACTIONS DUE TO INCOMPATIBLE AGGLUTININS

These are indeed rare and it is doubtful if they occur without other complicating factors. Reactions attributed to anti-A or anti-B agglutinins have been reported in the literature but the cause is considered somewhat controversial. It is certainly difficult to incriminate incompatible plasma agglutinins as the sole cause. The significance of these substances in plasma has been discussed on page 354 and in group O whole blood on page 245. Although specific A and B substances[15] may be added to unpooled plasma or group O blood to neutralize the anti-A and anti-B agglutinins if desired, there is not good evidence that even relatively high-titered incompatible agglutinins are a serious risk. Two recent papers have attributed death to this cause. Morgan and Lumb[14] reported an apparently typical fatal hemolytic reaction in a group A patient receiving group O blood. The anti-A titer of the donor's plasma was 1/4096. However, it must be noted that this was the last of several transfusions in a seriously ill patient. Alberton[1] reported another fatal reaction, apparently hemolytic, in a group A patient receiving 100 ml. of unpooled plasma with an anti-A agglutinin titer of 1/900. This was also the last of a series of transfusions in a patient who was seriously ill with extensive carcinoma. Neither of these reports, nor the few earlier ones, present entirely acceptable evidence that the possible danger warrants the routine use of the A and B specific substances in all transfusions of group O blood or unpooled plasma when the recipients belong to other blood groups. Nor, by the same token, is it necessary to use only blood or plasma of homologous group, even when the titer of the donor's incompatible agglutinins is more than the suggested safe limits of 1/256 to 1/500. In many of the other reports, such as that of Polayes and Squillace,[2] the reactions seem to be due quite evidently either to pyrogens or an Rh incompatibility, although neither was adequately investigated. In this connection, Weinstein reported his experience with 1500 transfusions of unpooled plasma.[7] The total reaction rate was 1 per cent; only one reaction, not severe, was not obviously due to pyrogens or allergy and in that the possibility was considered of incompatible agglutinins, although there was no supporting evidence.

Another cause of group-specific reactions to plasma has been advanced by Levine and State,[4,5,11] who attributed a great majority

pain in other locations, palpitation, anxiety and sweating. Such symptoms are most likely psychic,[4] and are often observed in the course of any special test or procedure involving intravenous injection.

Circulatory Overload. The circulation may be overloaded, resulting in cyanosis and dyspnea with or without frank pulmonary edema, by the too rapid transfusion of plasma, particularly in those patients who have evident or potential cardiac insufficiency or an impairment of the pulmonary circulation because of infection or chronic disease (p. 270). Good clinical judgment is imperative in avoiding such situations.

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with impairment of renal function, in severe shock. However, under possibly less critical circumstances, the amount of merthiolate in 10 liters of plasma has been given without evidence of renal damage.[4] The possibility of a severe or fatal reaction due to an idiosyncrasy to mercury must always be considered.

EMBOLISM

Blood plasma must always be filtered (p. 389) at the time of administration to remove particulate matter large enough to cause significant vascular embolism. Serious reactions and deaths have undoubtedly occurred from failure to observe this rule. Mayner[19] has reported one such death due to multiple small pulmonary emboli found at autopsy after the transfusion of unfiltered plasma.

MISCELLANEOUS REACTIONS

Tetany. This has been observed and commented upon as a contraindication to the use of any large amount of citrated plasma. On the basis of experiments on dogs, Ivy[6] stated that the rapid administration of 1500 to 2500 ml. of plasma (7.5 to 20 gm. of sodium citrate) might be fatal. Muirhead and Hill[13] observed tetany as a complication of the rapid administration of concentrated plasma in dogs. This plasma actually contained 2 per cent citrate and the tetany could be prevented by the use of calcium chloride. Both of these experiments were actually not comparable to the ordinary use of plasma in man, and Allen[5] reported on the massive administration of plasma in human patients as follows: Nine patients (seven weighing less than 154 lbs.) received a total of 2 gm. or more of sodium citrate in plasma without incident. He pointed out that, while this was more than the lethal dose calculated on the basis of animal experimentation, the period of time to administer the plasma was customarily considerably longer. For example, to duplicate the situation causing tetany in dogs, it would be necessary to give a 154 lb. man 4 liters of citrated plasma in five minutes. Tetany was not observed in dogs when the rate of plasma administration was comparable to that used in therapy.

Symptomatic Reactions. A variety of miscellaneous symptoms have been observed during or after plasma transfusion and have been attributed to the plasma. Probably the plasma, or a specific substance, has nothing to do with such symptoms. They include such manifestations as: nausea, vomiting, transient back pain and

CHAPTER 21

Red Cell Suspensions and Derivatives

By John B. Alsever

RED BLOOD CELL SUSPENSIONS
RED CELL PASTE AND POWDER
SOLUTIONS OF HEMOGLOBIN
SOLUTIONS OF GLOBIN

In spite of the fact that suspensions of erythrocytes were used successfully for transfusion in World War I,[23] this method of red cell replacement was not employed again to any extent until after World War II had begun. At this time the preparation of plasma for use by the armed forces in England and the United States had made large amounts of red cell residue available as a by-product. The wartime use of the red cells established that, under specific conditions, they may be employed quite satisfactorily as suspensions for transfusion and as a paste or powder for the dressing of wounds. In the search for blood substitutes, the red cells have also been employed as a source of hemoglobin and, more recently, for the preparation of globin, both of which have been administered experimentally in colloidal solution as substitutes for blood plasma.[14,20]

RED BLOOD CELL SUSPENSIONS

The use of red cell suspensions for transfusion during World War II was first reported by MacQuaide and Mollison,[32] who recommended 50 ml. of 8 per cent dextrose in isotonic saline as the suspension fluid. Since red cell residue was rather widely available as a by-product of plasma preparation, the employment of suspensions for transfusion spread rather rapidly during the next few years.[1,2,3,4,5,6,7,8,28,29,30,31,33,34] Cooksey, Thalhimer, and Taylor[4,6,7] developed a technique for the utilization of red cells salvaged from the preparation of plasma in the nation-wide blood donor program of the American National Red Cross, which made it possible as a major transfusion method. The centrifuged cells were returned to certain blood donor centers from the processing

16 Lozner, E L , Lemish, S., Campbell, A S., and Newhouser, L. R.: Preservation of normal human plasma in the liquid state; clinical, chemical and physico-chemical studies during three years of storage at room temperature. Blood 1:459, 1946

17 Lozner, E. L., and Newhouser, L. R.: Preservation of normal human plasma in the liquid state; statistical study of 1751 administrations. J. Clin. Investigation 23:343, 1944.

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19. Mayner, F.: Death from transfusion of plasma J.A.M A. 116:2015, 1941.

technique has been employed to prepare the plasma, the cells are not suitable for use (p. 366). In citrated blood the red cells may be used safely within five days after the collection of the blood. If the blood has been collected in one of the dextrose preservative solutions, the cells may be used safely for the duration of the storage period permissible for whole blood in the solution employed. If the red cells are to be salvaged for transfusion after aspiration of the plasma, particular care must be taken to preserve the sterility of the cell layer.

Containers. If commercial vacuum-type equipment is employed, the cells must be aseptically aspirated at once into a new container, either empty or with the desired preservative, since the seal of stoppers of these bottles is destroyed in aspirating the plasma. The technique of aspiration is identical to that described on page 368.

If reusable equipment is employed, the aspiration of the plasma should be carried out in a closed, sterile room. When the plasma has been removed, the two-hole stopper of the blood bottle is taken out and the container is promptly resealed with a sterile, solid stopper.

Blood Grouping and Pooling. The blood group, the Rh type (if determined), serologic findings, and identification data, obtained at the time the blood was collected, are accurately transcribed on the tag or label affixed to the bottle, if a new container is employed. Cells of the same blood group may be pooled if desired, but the group should have been proven by duplicate determinations in such cases (p. 140).

Sterility Test. Culturing of the red cell suspensions is not practicable as a routine measure and introduces an additional opportunity for accidental contamination. However, it is usually desirable to check sterility initially as a control of the technique employed, and as a periodic check at suitable intervals. Follow the method described on page 370.

Storage

After separation from the plasma, the red cells should be immediately returned to the refrigerator at 5° to 10° C. In general, suspensions may be stored before use as long as citrated or preserved whole blood under the same conditions. The maximum safe period depends, of course, on the preservative solution employed, and the storage period dates from the collection of the blood from the donor.

Citrate-Saline. Citrated cells, with or without added saline, may be stored up to five days.

Citrate-Dextrose. Citrated cells from 500 ml. of whole blood

laboratories, where they were prepared for use and distributed to nearby hospitals, both military and civilian. Some 25,000 suspensions were given. The cells were mostly resuspended in saline, but some were preserved in corn syrup or certain of the whole blood preservative solutions. Studies of over 5000 such transfusions showed good clinical results with adequate red cell survival after transfusion, as demonstrated both by Ashby's agglutination technique and by study of cells tagged with radioactive iron (p. 311). The reaction rate was satisfactorily low. Because of the interest of the armed forces in blood preservation, numerous other studies were carried on under the auspices of the National Research Council, both to evaluate the existing techniques and to search for better ones. The results of some of these have been recently published and include some data on red cell suspensions. These workers[24,25,26,27] studied the preservation of red cells in a number of solutions and recommended safe limits of storage for certain of them, on the basis of the radioactive iron technique (p. 225), as follows: (1) undiluted red cell residue from blood collected in ACD solution may be stored up to twenty-one days; (2) red cell residue from citrated blood may be stored up to ten days, if preserved by the addition of ACD solution; (3) red cell residue from citrated blood may be stored for at least ten days, if preserved by the addition of 10 per cent corn syrup. The necessity for prompt and adequate refrigeration and the addition of dextrose are emphasized by these studies. Experimental preservative solutions studied by these workers have included the use of plasma fractions and globin. The available evidence does not indicate that they materially improve red cell preservation.

The use of cell suspensions has proven to be quite adequate in the treatment of chronic anemia. Since the cells can be given in a concentrated suspension, anemia can be corrected rapidly by the continuous or sequential administration of several suspensions, and the volume of fluid injected is reduced to about one half that required with whole blood. This procedure results in conservation of the supply of whole blood for other cases in which both cells and plasma are essential. Concentrated suspensions have been of particular value where anemia exists in infants or in patients with cardiac failure to whom the volume of fluid administered should be kept as small as is possible.

Preparation

Sedimented red cells are suitable for transfusion only if the preservation has been adequate. The conditions are the same as for whole blood (Chap. 13). However, if the room temperature

It has been observed that topical application aids the growth of new tissue in open joints, infections, burns, and ulcers (especially the trophic, decubitus, and diabetic types). The healing effect is not limited to the cells since the application of a plasma dressing has a like action.

PASTE

This is the simplest to prepare, but the rather thick liquid preparation is not so convenient to use as the powder or ointment. The cells from several blood collections are aseptically pooled, groups may be mixed, and stored in the refrigerator for ten to twenty days to permit the cells to disintegrate. The paste is then ready for use. Such a preparation may be kept for a more or less indefinite period under refrigeration.

POWDER

This is prepared by drying the cells under aseptic conditions. The cells can be frozen and desiccated in the plasma drier, or spray dried in the type of apparatus used for preparing powdered milk. The former method results in a cake of dried material which must be powdered, while the latter dries the cells as a powder. The powdered material is placed in sterile glass containers which are then sealed and stored indefinitely.

OINTMENT

Muether[3] prepared an ointment by mixing the powdered cells with a base which has the following formula:

Cetyl alcohol	15.0 gm
White wax	1.0 gm.
Propylene glycol	10.0 gm.
Sodium laurel sulfate	2.0 gm.
Water	72.0 ml.

He has found the preparation to be very satisfactory. It apparently prevents the painful sensation sometimes caused by application of the powdered cells alone.

SOLUTIONS OF HEMOGLOBIN

Solutions of hemoglobin have been prepared by several workers and used experimentally as a blood substitute in animals and in man.[15,16,17,18,19] For example, Amberson[17,19] showed that animals lived many hours in an apparently normal state when the circulating blood was replaced by oxyhemoglobin solution, salt and sugar. However, Lamson[15] in further animal experiments found that

with 250 ml. of a dextrose preservative added (p. 438), may be stored up to ten to fourteen days. The cold solution should be added to cold cells which have been no more than twenty-four to forty-eight hours in citrate solution.

Citrate Corn Syrup. Citrated cells, from 500 ml. of whole blood, with 250 ml. of 10 per cent corn syrup added, may be stored as long as twenty-one days, according to Thalhimer and Taylor,[4] who developed this technique with the Red Cross blood donor program. They had rather extensive experience with it, carefully studying some 700 transfusions. The cold solution should be added to cold cells which have been no more than twenty-four to forty-eight hours in citrate solution. The composition of the corn syrup used was: 17.7 per cent dextrose, 16.8 per cent maltose, 16.2 per cent higher sugars, 29.6 per cent dextrins, and 19.7 per cent water.

Cells from Whole Blood Preservative Solutions. Cells from blood originally collected in one of the dextrose preservative solutions may be used up to the outdating period permissible with the particular solution. It is not necessary to add any diluent. Storage of the concentrated cells is preferable.

Administration

Red cell suspensions are administered to the patient with the same precautions and in the same manner as whole blood (p. 250): (1) The patient's blood group and Rh type are determined. (2) The suspension is examined for color and the presence of clots. A dark red or violet color and/or clotting is presumptive evidence of contamination and such a suspension should be discarded. (3) The selected cell suspension is gently mixed and a 5 ml. sample withdrawn for: (*a*) crossmatching with the patient's serum, (*b*) centrifugation to check for excessive hemolysis and rapid osmotic fragility test (p. 198).

If all tests are satisfactory, the suspension is promptly administered to the patient through a standard filter set without allowing it to come to room temperature, or warming it at the time of administration.

RED CELL PASTE AND POWDER

The use of red cells as a paste, powder, or in an ointment for the dressing of wounds has been described by several authors. Application of the paste has given good results[9,12] as has the use of dried, powdered cells[10,11,13] and an ointment containing dried cells.[8]

modified globin in the treatment of hypoproteinemic states, since it is difficult and expensive to obtain the very large amount of plasma which is required. He states that it is relatively easy to prepare and give up to 700 gm. of globin to a patient with severe hypoproteinemia. This is the equivalent of about 13 liters of plasma, or fifty-three blood donations. He reports good results even in severe cases with reduced protein reserves and liver and kidney damage, in which the use of amino acids is of little value and sufficient plasma impossible or impractical to obtain and administer. It seems evident that further work with globin is indicated and that, if Strumia's findings are confirmed, globin solutions might well find a place among the useful blood derivations.

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hemoglobin solutions would support life in shocked animals for *only a few hours, and then only if given before severe shock* developed. He used a stable 7 per cent solution, which is isotonic with plasma. Pertinent observations were that the rise in blood pressure was more than would be expected by the volume of fluid replacement, the pulse pressure increased markedly, and the rate of metabolism rose more than 100 per cent.

Hemoglobin solutions, therefore, are not a satisfactory blood substitute and their use clinically is not now warranted. Their use did not support life in shocked animals for more than several hours. The metabolic rate was increased tremendously, which is most undesirable in shocked cases. Moderately severe reactions have resulted in human beings when hemoglobin solutions were given in amounts too small to be of therapeutic value in the treatment of shock. Furthermore, injection of the hemoglobin solutions cannot be reconciled with the facts known about its toxicity (p. 274.)

SOLUTIONS OF GLOBIN

Globin, the protein component of hemoglobin, constitutes 96 per cent of this compound. It can be chemically separated quite easily by treating oxyhemoglobin with acid. However, some is denatured in the process and is therefore toxic. Such material must be removed to prepare solutions of globin which are fit for intravenous use. Strumia[21,22] has recently developed a modified globin which he has successfully employed experimentally in the treatment of shock and hypoproteinemia in man.

The modified globin was administered as a 1.8 to 6.8 per cent solution at pH 7.4. It is estimated that 12 gm. of globin is osmotically equivalent to 300 ml. of plasma. The viscosity in such solutions is less than that of plasma. Eighty-five per cent of this globin preparation has a molecular weight of about 34,000 and the molecule is quite symmetrical. The yield is about 250 gm. per 1000 ml. of packed red cells. The product is stable in isotonic saline solution. *Agglutination or precipitation is not caused by admixture* with blood or plasma. The injection of globin produces an increase in blood volume lasting twenty-four to 120 hours in both normal subjects and hypoproteinemic patients. Preliminary animal studies showed it to be nontoxic and nonallergic. This was confirmed by skin tests and repeated injection in human experiments. Over 210 injections have been given to 108 persons. The largest single dose was 57 gm., the largest amount given in twenty-four hours was 60 gm., and the largest total dose was 192 gm.

Strumia has been especially interested in the apparent value of

CHAPTER 22

Plasma Derivatives

By JOHN B. ALSEVER

Although most of the components of plasma had been known and isolated chemically for some years, it remained for E. J. Cohn and his coworkers to develop a method, based on arbitrary physicochemical methods of separation, whereby plasma could be separated into fractions which were suitable for parenteral use. Each fraction may contain one or more compounds. The work was carried out with human blood made available through the wartime Red Cross blood donor program, with the cooperation and support of the U. S. Navy and the Committee on Medical Research of the U. S. Office of Scientific Research and Development. Refinements of techniques are still being developed, particularly in an effort to isolate more of the serum proteins in pure form. Some fractions are of potential therapeutic value and others of value only in furthering the knowledge of the composition and functions of the blood and other organs of the body. The greater the number of useful products eventually developed with this process, the cheaper will be the cost of preparation of each individual product. Should the fractions become generally available at a reasonable cost, the ability to use only the particular component indicated in a certain condition will conserve the amount of blood required for adequate thereapy whenever whole blood or whole plasma is not required. Since these fractionation methods are applicable to the biologic industry as a whole, their use should result in the preparation of purer and better animal sera, vaccines, and tissue extracts.

FRACTIONATION OF PLASMA BY THE COHN METHOD

The fractionation process is carried out in a cold room at −5° C. A small number of technicians can process as many as 10,000 bleedings per week into the crude fractions. Preparing and testing

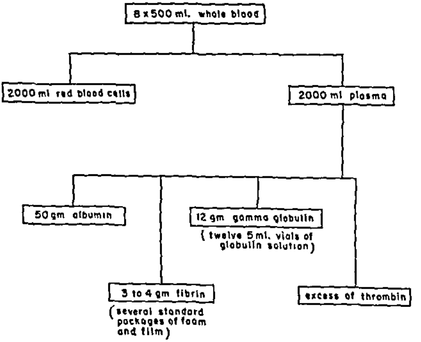
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TABLE XXIV

Content of Plasma Fractions*

Fraction I...	[illegible]
Fraction II..	[illegible]
Fraction III-1 .	[illegible]
Fraction III-2	[illegible] products) and one of the components of complement
Fraction III-3..	Plasminogen (precursor of plasmin) and "fibrinolytic" enzyme
Fraction III-0 .	Rich in lipoprotein (cholesterol, carotene, vitamin A), beta globulin
Fraction IV-1	Lipoprotein, alpha globulin
Fraction IV-4	Enzymes and hormones (hypertensinogen and thyrotropin), subfractions yield serum esterase and iron binding protein
Fraction V. . .	Albumin
Fraction VI	Salts, small amounts of protein, and small organic molecules

AMOUNTS OF USEFUL FRACTIONS.



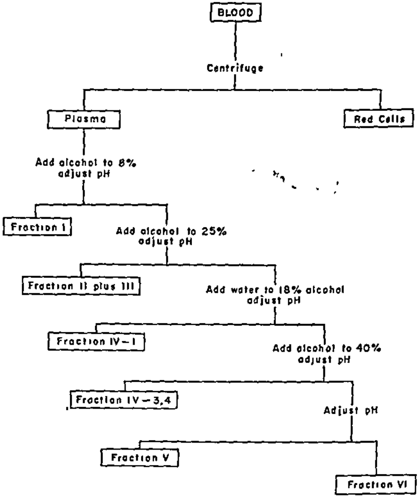
Adapted from Janeway, C. A: Clinical Use of Products of Human Plasma Fractionation. New York State J. Med. *47*: 1357, 1947.

content of each of the fractions obtained by Cohn, and the diagram above the approximate relationship between the amount of original blood and the quantity of useful products obtained. In a large scale program the cost of fractionation and the preparation of the finished products is not prohibitive. The cost has been estimated to be about $5.00 per 500 ml. of blood, on the basis of the experience gained by 1945 in the national wartime program for the armed forces.[2,7,12,13,17,20,21,22]

* From Cohn, E. J.: Separation of blood into fractions of therapeutic value. Ann Int. Med. 26·341, 1947.

the final products takes considerably longer. Each protein fraction is precipitated in a different concentration of ethyl alcohol under specific conditions of temperature, pH, ionic strength and protein concentration. The fluid portion is removed by centrifugation, leaving the precipitated protein as a paste. The paste is quick

PLASMA FRACTIONATION.



frozen and dried by the same process that has been described for plasma. Because the entire process is carried out below the freezing point of water, the alcohol does not denature the plasma proteins, and bacterial growth is inhibited. The several fractions are stored in the dried form pending the actual preparation of the various products for clinical use. The accompanying diagram shows in outline form the alcohol concentrations which have been employed for the initial separation into the major fractions, some of which are further separated by sub-fractionation. Table XXIV shows the

few milligrams of material. The preparations tested so far vary greatly in potency and, in some cases, the crude fraction I is much more effective than the purified material. It is possible, therefore, that there may be two or more factors responsible. The usual recommended initial dose of fraction I has been 400 mg., dissolved in 20 ml. of salt solution and given intravenously. However, the clinical result is the guide to the amount required and second or third doses may be given at thirty minute intervals if the response is not satisfactory. Larger amounts frequently do not further effect the magnitude or duration of the fall in the blood clotting time of hemophilic patients.

Immune Serum Gamma Globulin

Gamma globulin evidently contains most of the antibodies which are present in the pooled plasma of normal persons who have had the common infectious diseases. These antibodies include: diphtheria antitoxin, scarlatinal antitoxin, complement-fixing and mouse-protective antibodies to influenza A virus, and agglutinins for the H antigen of the typhoid bacillus. The present preparation is a 16.5 per cent solution of the protein fraction, more than 95 per cent of which is gamma globulin. It must be given intramuscularly since it produces reactions when given by vein. It is hoped that a modification of the technique of preparation can be found which will eliminate this disadvantage. The antibodies present in the original plasma are concentrated about twenty-five times in the immune serum globulin described.[2,17,21,23]

Therapy of Measles. Gamma globulin is the best and most reliable agent for the prophylaxis and modification of measles (rubeola). Janeway[9] reported a careful study of 1843 cases with reactions in only 1.7 per cent. About one half the reactions consisted of some local soreness and the rest were characterized by a little fever. There was one anaphylactoid reaction. The clinical results were excellent. A dose of 0.1 ml. per pound (0.22 ml. per kg.) was used for complete protection and 0.02 ml. per pound (0.044 ml. per kg.) for modification of the course and severity of the disease. The study reported by Greenberg and Rutstein[10] also showed excellent results. In a series of 814 children, six months to six years of age, who received 2 ml. of the gamma globulin solution after exposure to measles, 78 per cent had no measles; the rest had a modified disease, of which 92 per cent were mild, and reactions were rare. Simultaneously, ninety similar persons with exposure were protected with 5 ml. of placental globulin, but 23.3 per cent had severe measles, 37.7 per cent had a modified disease, and only 38.9 per cent were completely protected. In this series 41 per cent

FIBRIN FOAM AND THROMBIN

The foam is prepared by mixing the fibrinogen with thrombin and beating it with air as it coagulates, to attain the required spongy consistency.[21] It is then cut to the desired size and shape, dried and sterilized by heat. The dry foam is stable and rather brittle. To prepare it for use it is soaked in a solution of thrombin, which makes it flexible. When applied to a bleeding area, the blood enters the foam and is promptly clotted; the foam thus initiates and forms part of the clot. Since it is human protein and absorbable, the foam can be left *in situ* with only a minimum of resulting tissue reaction. Fibrin foam has had its widest application in neurosurgery.[18,19] Gelatin foam possesses quite similar properties (p. 458).

FIBRINOGEN AND THROMBIN

Thrombin, prepared by the conversion of prothrombin, and fibrinogen are stable when dried from the frozen state.[21,23] They have proven useful as a means of forming a coagulum around renal calculi, so that they may be completely and easily removed at operation, and as a physiologic adhesive in the application of skin grafts.

FIBRIN FILMS, TUBES, ETC.

Fibrin is a natural plastic and a number of objects can be prepared from fibrinogen by varying the conditions of clot formation and subjecting the fibrin to different types of chemical, mechanical and thermal treatment.[21] Fibrin film, as it is prepared for use, is a tough, elastic, semitransparent membrane and has been used as a substitute for the dura mater in operations on the brain. When employed for this purpose it is slowly absorbed and is replaced by connective tissue without producing adhesions between the brain and its coverings.[18,19] Fibrin has also been processed into tubes, plates and other forms for experimental trial in various surgical procedures (See also *Polyethylene*, p. 458).

ANTIHEMOPHILIC GLOBULIN

This substance is precipitated with fibrinogen in fraction I.[21,23] The entire fraction has been successfully employed experimentally in the control of bleeding in hemophiliacs.[1,2,15] It has been estimated that 200 to 600 mg. of fraction I is about equivalent to 80 ml. of fresh plasma in bringing the coagulation time back to normal. Minot[15] reported that the active substance can be isolated by heating fraction I to 56° C. for five minutes and filtering. The filtrate is active and contains no fibrinogen. He stated that it is probable that the antihemophilic property is contained in only a

SERUM ALBUMIN

Albumin[8,9,11,16,21] constitutes 55 per cent of the total plasma protein and contributes the major portion of the osmotic pressure of plasma. In solution it is also less viscous, more soluble, and more stable than most of the other plasma proteins. It has been prepared for use as a concentrated solution, both with added salt (0.3 M concentration) and with a low salt content (less than 0.1 M concentration). While the latter is preferable for the replacement of plasma protein in the presence of edema, the former may well give better results in the treatment of shock. A twenty-five per cent solution of albumin is standard since this has a viscosity about equal to the whole blood. One hundred milliliters of this solution is the osmotic equivalent of 500 ml. of plasma. It is stable at ordinary temperatures, can be added to crystalloid solutions if more fluid is desired, and provides a substitute for plasma which is compact and easily administered. The transmission of homologous serum jaundice has not been reported with the use of serum albumin.

Clinically albumin solution intravenously is effective in the treatment of shock. Its use in the treatment of nephrosis, hepatitis, and hepatic cirrhosis is still experimental. When concentrated albumin is employed in the treatment of shock complicated by dehydration, added fluid must be given in sufficient quantity to restore the fluid balance, if optimal results are to be obtained. The initial dosage recommended in shock is 100 to 200 ml. (25 to 50 gm.), the osmotic equivalent of 500 to 1000 ml. of plasma. This dose may be repeated as necessary. The need for whole blood must, of course, be met as is the case when plasma is transfused. If plasma loss has been severe, replacement by albumin alone may leave the patient with a seriously depleted supply of serum globulins. Concentrated albumin has been employed experimentally to restore the depleted serum protein level in cirrhosis and nephrosis, although rather large amounts are required and its beneficial effect appears to be only temporary. In nephrosis its use is not always effective, and the massive proteinuria which follows its administration may in itself produce renal damage. It may be more useful in acute but reversible states, since injection has been reported to be of great benefit in hepatitis when the plasma protein level is low. It is possible that albumin may prove to be of value in the treatment of acute renal failure as a means of maintaining a normal serum protein level without elevating the level of nonprotein nitrogen.

COMMENT

Although the cost of plasma fractionation is not prohibitive (p. 447 and 507) in large scale production, it is relatively expensive

had reactions. The control series showed a disease incidence of 83 per cent among unprotected contacts, in 31 per cent of whom the measles was severe. Stokes[14] reported success in modifying measles in the stage with Koplik spots by the use of large doses. He also indicated suggestive evidence that the administration of gamma globulin after exposure to the disease will prevent the development of German measles (rubella).

Therapy of Infectious Hepatitis. The use of gamma globulin has been reported to be effective in the prophylaxis of epidemic infectious hepatitis but of no value in the treatment of the disease.[14] There is only questionable evidence[5,6] that the use of globulin will prevent or modify the onset of homologous serum jaundice and therefore, is not recommended.

Therapy in Other Diseases. The value of immune serum globulin is being investigated in other diseases, especially scarlet fever. Its use has proven unsatisfactory in the prophylaxis or modification of mumps, chicken pox, and anterior poliomyelitis.[14,17] In respect to the latter disease, the study of Perkins and Bahlke[4] demonstrated that gamma globulin was of no value. One hundred and eleven patients, twelve years of age or under, hospitalized with preparalytic poliomylitis were observed for a period of six months. Half of these, fifty-six, were immediately given large doses of gamma globulin, with alternates used for controls. The dose varied from 20 to 100 ml., depending on weight, the equivalent of 500 to 2500 ml. of plasma. There was no detectable benefit (see p. 424 for a full discussion).

The preparation of gamma globulin from convalescent plasma would yield a very potent material for use in a specific disease and the high antibody content does not affect the usefulness of the other fractions prepared from such blood. This has been done with hyperimmune pertussis plasma (p. 421) and convalescent mumps plasma.[17]

The Isohemagglutinins

Potent, dry, standardized blood grouping preparations can be produced by pooling the plasma from a single blood group.[16,21] The anti-B material is obtained from group A bloods. The best anti-A product is obtained from group O bloods absorbed with group B red cells, whereas group B blood is not so good a source. As a matter of fact, this is most fortunate, since A and O are the commonest groups and have about equal incidence in Caucasians, while the B blood obtained in a donor recruitment program is sufficient to provide enough red cells for absorption of the O plasma. Similarly, by pooling bloods with a high titer of anti-Rh, a potent Rh-typing material may be prepared.

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    I. Characterization of the protein fractions of human plasma;
    II Electrophoretic and ultracentrifugal studies of solutions of human serum albumin and immune serum globulins;
    III. Amino acid composition of plasma proteins;
    IV. Study of the thermal stability of human serum albumin;
    V. Influence of non-polar anions on the thermal stability of serum albumin;
    VI. Osmotic pressure of plasma and of serum albumin;
    VII. Concentrated human serum albumin;
    VIII. Clinical use of concentrated human serum albumin in shock and comparison with whole blood and with rapid saline infusion;
    IX. Treatment of shock with concentrated human serum albumin, a preliminary report;
    X. Concentrations of certain antibodies in globulin fractions derived from human blood plasma;
    XI. Use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prophylaxis and treatment of measles;
    XII. Use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prevention and attenuation of measles;
    XIII. Separation and concentration of isohemagglutinins from group-specific human plasma;
    XIV. Appraisal of isohemagglutinin activity;
    XV. Proteins concerned in the blood coagulation mechanism;
    XVI. Fibrin clots, fibrin films, and fibrinogen plastics;
    XVII. Note on absorption rates of fibrin films in tissue;
    XVIII. Fibrinogen coagulum as an aid in operative removal of renal calculi;
    XIX. Note on use of fibrinogen and thrombin in surface treatment of burns;

when compared to the cost of achieving similar clinical results with blood products prepared by other methods or with substitutes for certain of the fractions. Gelatin foam and thrombin (animal or human) will give results equivalent to human fibrin foam and thrombin (p. 458). A new synthetic plastic, polyethylene, may be as satisfactory as human fibrin films, tubes, and so forth (p. 458). Dried blood plasma, three or four times concentrated, is almost as easy to administer and will probably give results equivalent to those obtained with antihemophilic globulin, immune serum globulin, and serum albumin (Chap. 2). Anti-A and Anti-B grouping sera and Rh typing sera can be prepared more simply and more satisfactorily by other methods (p. 132). Such techniques yield a more avid and potent sera which, when dried from the frozen state, are as stable as the preparations produced from large pools of plasma by the Cohn plasma fractionation method.

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CHAPTER 23

Plasma Substitutes

By John B. Alsever

There has been considerable interest in the possibility of developing a satisfactory substitute for human blood plasma. To date, none of the substances employed for this purpose has proven to be entirely satisfactory. For example, gelatin, which is the best available, is only temporarily effective in relieving shock, and blood or plasma must be given subsequently for lasting benefit. However, the time, expense, and effort involved in maintaining an adequate supply of blood plasma continues to encourage research in this field.

GELATIN

Solutions of gelatin, satisfactory for intravenous use, can be prepared by proper technique. They are sterile, nonpyrogenic, nontoxic in therapeutic amounts, and free from antigenicity. Animal gelatin prepared from bone is usually employed for this purpose. It is an incomplete protein, since it does not contain all of the essential amino acids and, therefore, in itself cannot supply the protein requirements of the body.

Physical Characteristics

During World War II *gelatin preparations were rather extensively* investigated as possible substitutes for blood plasma. The work was carried out under the auspices of the National Research Council. It was found that gelatin possessed more nearly satisfactory physicochemical characteristics for this purpose than any of the proposed substitutes except globin (p. 442), which was still in the experimental stage at the close of the war. The natural undegraded gelatin molecule is about thirty times longer than it is wide and has a molecular weight approximately 150 per cent that of serum albumin. The albumin molecule is twice as wide as gelatin but only

XX. Development of fibrin foam as a hemostatic agent and for use in conjunction with human thrombin;
XXI. Use of fibrin foam as a hemostatic agent in neurosurgery, clinical and pathological studies;
XXII. Fibrin films in neurosurgery, with special reference to their use in repair of dural defects and in prevention of meningocerebral adhesions;
XXIII. Effects of feeding possible blood substitutes on serum protein regeneration and weight recovery in the hypoproteinemic rat. J. Clin. Investigation 23:417–606, 1944.

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stream interferes with blood grouping and crossmatching by causing rouleau formation (pseudoagglutination) and increases the sedimentation rate of the red cells. It also displaces the plasma proteins from the blood stream into the tissues. All these effects are reversible; they disappear as the gelatin is excreted from the body, and they do not necessarily make the use of gelatin solutions undesirable (see following sections).

Experimental Findings

Robscheit-Robbins and Whipple,[7] in a studying of the value of gelatin solutions for the treatment of dogs with depleted blood proteins, found no immediate toxic or anaphylactoid reactions and believed that the gelatin might contribute something to the production of new hemoglobin and plasma protein. The use of large amounts over a period of one to two weeks usually caused serious inhibition of the synthesis of blood protein, intoxication, weight loss and, at times, death. Toxic effects were not observed when gelatin was used in smaller dosage, 1 to 2 gm. per kg., for two or three days, with added amino acids. These findings indicate caution in the use of repeated large doses in humans. Brunschwig[2] observed that gelatin has some nutritive value in animals and found similar effects in a few patients, stating that certain types of gelatin were at least partially utilized. Fletcher,[4] Koop,[10] Popper,[6] Ivy,[9] and others [2,7,8,11,12] have observed satisfactory effects in the emergency treatment of shock in both animals and human beings. Jacobsen,[11] studying gelatin excretion in normal subjects, with the more degraded gelatin solution, found that the highest rate of excretion occurred during the first hour and that 91 per cent of the injected gelatin was recovered in the urine in twenty-four hours. Lowell and his coworkers[12] prefer a less degraded gelatin solution because it remains in the blood stream longer. It is, however, more difficult to administer since it is a solid gel at room temperature and must be heated before use. This group confirmed an earlier observation that thrombosis of the injected vein is common when phenyl mercuric borate has been added to the solution as a preservative.

Clinical Use

The temporary effectiveness of gelatin solutions has been discussed. Another important problem which has been mentioned in the use of these solutions is the fact that marked rouleau formation occurs which ordinarily makes blood grouping and crossmatching most difficult for about twenty-four to forty-eight hours while the gelatin remains in the circulating blood. This could be a most serious problem in a patient with shock to whom gelatin had been

one fifth as long. Two preparations of gelatin which possess an average molecule length approximating that of serum albumin were given principal attention. One is slightly degraded (autoclaved for about twenty minutes), has an average molecular weight of about 35,000 (albumin has a molecular weight of 69,000), an average molecule length nearly twice that of albumin, is a solid gel at room temperature and has to be heated before use. The other is further degraded (autoclaved for about eighty minutes), has an average molecular weight of about 20,000, an average molecule length slightly less than that of albumin, and is liquid at room temperature. However, both solutions are composed of gelatin particles of many different sizes, from very small to those of original length. The width of the gelatin molecule is not altered by degradation. These data were calculated largely from ultracentrifuge and viscosity studies.

The long molecules are responsible for the high viscosity of gelatin solutions and for increasing the sedimentation rate of the red cells when gelatin is injected. The greater the degree of degradation, the fewer the number of long molecules and the less the viscosity. Although the reduced molecular size of the degraded solution increases the effective osmotic pressure, it also permits a more rapid diffusion of the gelatin molecules through the membranes of the body. Therefore, in order to obtain a satisfactory viscosity and osmotic pressure, duration of effectiveness in the body must be sacrificed to some extent. Solutions containing 4 to 5 per cent of these two gelatin preparations were found to possess an osmotic effect approximately equivalent to that of isotonic plasma.

Fate in the Body

Both the less and the more degraded gelatin solutions are rapidly excreted from the body in the urine. Only very little of the more degraded type remains in circulation at the end of twenty-four hours. The less degraded type is excreted about half as rapidly. The evidence indicates that almost none of the gelatin contributes to body nutrition.

Toxicity

No toxic reactions have been observed with the use of properly prepared solutions of gelatin and there has been no evidence of sensitization after repeated dosage. Popper[6] was unable to find any evidence of deposition of gelatin in the body and but little evidence of any tissue change in a careful study. Although the injection of these solutions is effective temporarily in relieving shock, there are certain undesirable effects. The presence of gelatin in the blood

In addition, it can be readily made into flexible tubes and thin, pliable sheets. Ingraham and his coworkers[14] recently published their results with the use of this material in experimental brain surgery in animals. Implantation of the plastic in the brain did not produce any significant tissue reaction. A film used as a dural substitute produced no more tissue reaction than fibrin film. However, the polyethylene film behaved like tantalum foil in respect to the formation of adhesions, gradually becoming enveloped by a thin layer of cells which subsequently became adherent to the adjacent brain tissue. It was concluded that, while this synthetic will probably not replace the use of fibrin film to prevent the formation of adhesions over areas of damaged cortex, its ability to hold silk sutures securely should make it superior to fibrin film and tantalum foil as a dural substitute when the closure of dural defects must be water-tight.

OTHER PROPOSED SUBSTITUTES

Pectin

Solutions of pectin have been employed by several investigators [6,15,16,17,18,19] with clinical results quite similar to those obtained with gelatin in the treatment of shock, and with similar effects upon the red blood cells. There is, however, one most important difference. Although no immediate toxic effects have been reported, less than half of the injected pectin can be recovered in the urine, the rest is deposited in the tissues and organs of the body. Popper[6] found that the injection of pectin resulted in the deposition of a material, which closely resembled it, in various organs, including the spleen, kidneys, liver, and lungs. Although the clinical significance of the deposition of the pectin-like material is not known, it suggests that there may be late toxic effects and constitutes a definite contraindication to the further use of pectin solutions intravenously.

Acacia

The use of solutions of acacia as a plasma substitute originated during World War I and it has been employed considerably as an effective method of raising the osmotic pressure of the blood above the edema level in the treatment of nephrosis. It is given as a 6 per cent solution. The results [20,21] obtained with its use are similar to those which have been described with gelatin and pectin. Like pectin, it is deposited in tissues and organs of the body, especially the liver and spleen. The use of acacia as a plasma substitute is, therefore, likewise contraindicated.

given as emergency therapy. However, Koop[6] recently reported: (*a*) that the addition of 5 per cent dextrose to the cell suspension made from the patient's blood will reduce the psuedoagglutination and (*b*) that the use of 1 per cent glycine in isotonic saline for the cell suspension will abolish it. He found that neither the glucose nor the glycine had any effect on the accuracy or ease of blood grouping or crossmatching. It would appear, therefore, that the effect of gelatin on the behaviour of the red cells need no longer be a deterrent in the administration of gelatin solutions to patients who also will need transfusions of whole blood.

Isinglass (Ichthyocolla)

Fish gelatin was employed experimentally in Canada during World War II in the treatment of shock. The experience with its use, as reported by Taylor[14] and Pugsley,[13] was essentially similar to that described with the use of ossein gelatin. It was employed in concentrations of 4 to 7 per cent. The chief problem encountered was a relatively greater difficulty in preparing pyrogen-free solutions than was experienced with the ossein type.

Comment

Gelatin solution is the best of the plasma substitutes, but it is not the physiologic and metabolic equivalent of plasma. However, although its effect is temporary, it is far superior to any of the crystalloid solutions in the treatment of shock.

Gelatin Sponge

Properly prepared gelatin can readily be processed into a sponge-like form and employed, usually with thrombin, in precisely the same manner as human fibrin foam (p. 448). The thrombin may be either human or animal in origin. Bovine thrombin has been employed successfully. Jenkins[1] studied the clinical use of the gelatin sponge for surgical hemostasis and found that (1) it is absorbed in about five weeks; (2) it produces relatively little tissue reaction; and (3) it is effective as a hemostatic agent without thrombin, although the results are better with it. Pilcher[3] reported that the gelatin product is safe and efficacious and that it is easier to handle, less expensive, and more easily obtained than fibrin foam. His experience comprises 272 neurosurgical operations in 115 of which fibrin foam was also employed for comparison.

POLYETHYLENE

Polyethylene, a new synthetic plastic, is one of the very few substances in this group of materials which is well tolerated by tissues.

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Methyl Cellulose

Methyl cellulose has been suggested as a plasma substitute. However, experiments on dogs show that methyl cellulose is very toxic to the kidneys.[22,23] Large doses produce acute rènal failure, while the use of smaller amounts results in the development of chronic renal lesions which are pathologically similar to chronic glomerular nephritis in man. It seems quite evident that methyl cellulose should not be employed as a plasma substitute.

Animal Proteins

Colloidin Infusion. A substitute for human serum albumin, and presumably for plasma, has recently been proposed by Federov.[24] He reports good results with its use in 1000 patients with such conditions as burns and intestinal disorders. Preliminary animal experimentation showed no evidence of anaphylaxis or toxicity. However, the development of serum precipitin reactions in patients was observed, although there were no allergic manifestations. The formula given is: 7.0 gm. NaCl; 0.1 gm. KCl; 0.1 gm. $CaCl_2$; 30 to 40 gm. purified casein (milk); 2.5 gm. $NaHCO_3$; 1000 ml. H_2O. Its use has not been reported in this country.

Bovine Albumin. Considerable research was carried on during World War II, with bovine plasma, in an effort to obtain a nonantigenic animal protein derivative for use as a plasma substitute for the armed forces. The fractionation technique of Cohn (Chap. 22) was employed and the project was carried out under the direction of the National Research Council. Unfortunately, the effort was not successful. Even the most carefully prepared crystalline bovine albumin was found to retain some antigenic properties.

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the blood of donor and recipient, (6) the collection of blood from the donor, (7) the injection of the donor's blood into the recipient.

When transfusions of *fresh* blood are given, the procurement of the donor may take minutes or hours, depending on whether previous consent to give blood has been obtained, the geographical location of the donor when the call is made, the time of day or night when the occasion arises, and the health of the donor when the request is made. Many of these factors of delay may be eliminated, or the time reduced, by keeping a donors' registry but, even at best, much time is consumed *after the need for transfusion has arisen.*

When a donor's registry is maintained the determination of the blood group of the donor may be performed before the occasion for transfusion arises. If the blood group of the donor has not been determined previously, many persons may be tested before one of the proper group is found. This not only causes great delay but also results in the performance of many laboratory tests which are of no immediate use to the hospital or the patient.

The operation of a blood bank involves the use of *stored* blood. The storage of blood results in a redistribution of the elapsed time between the request for transfusion and the injection of the blood. In the use of *fresh* blood it may be necessary to perform all seven procedures *after* the occasion arises. In a blood bank the procurement of the donor, the determination of the donor's blood group, the tests of the donor's blood for evidence of syphilis, and the collection of the donor's blood are accomplished *before* the request for transfusion is made. With a donors' registry previous grouping and testing for syphilis can be performed but the elapsed time after the demand for transfusion never can approach the brief interval which is possible when banked blood is employed. The time factor precludes transfusions of fresh blood in many cases of acute hemorrhage whereas the promptness with which stored blood can be administered may be life-saving. Table XXV contains estimates of the time consumed in preparation for transfusion after the request for blood has been made.

Treatment of Acute Hemorrhage. The prompt availability of stored blood in a bank permits the treatment of patients with acute hemorrhage who would otherwise die of shock and exsanguination. Experience in World War II has shown that any delay in the treatment of hemorrhagic shock by the transfusion of whole blood adds greatly to the mortality.

Insurance Against Shock During Operation. The blood bank presents the facilities for furnishing the surgeon and the patient some protection against shock during elective operations. The blood

CHAPTER 24

Operation of a Hospital Blood Bank

By Elmer L. DeGowin

A blood bank may be defined as an establishment for the storage and exchange of human blood to be used for transfusion. The deposits and withdrawals of blood in the bank are credited and debited in much the same manner as financial transactions are recorded in a monetary bank.

ADVANTAGES OF A BLOOD BANK

Blood banks have been operated in the United States since 1937. The accumulated experience of many hospitals has demonstrated that there are several advantages which accrue by keeping a stock of blood which is readily available for use in transfusion.

Prompt Availability of Whole Blood

When a bank is operated, whole blood of the proper group can be transfused into the recipient within a few minutes after the need arises. This is in marked contrast to the situation in which fresh blood can be employed.

The time-consuming procedures which are necessary to transfusion may be listed as: (1) the procurement of the donor, (2) the determination of the blood group of the donor, (3) the performance of serologic tests for syphilis on the donor's blood, (4) the classification of the blood group of the recipient, (5) the crossmatching of

Convenience in Collection of Blood

When a bank is operated the blood may be collected at the convenience of the donor and the hospital staff, rather than at the unselected time of an emergency. Thus the services of the hospital personnel may be utilized most efficiently. Many donors are enabled to give blood who cannot be contacted during emergencies.

Increase in Use of Blood Transfusions

In hospitals in which blood banks have been established the number of transfusions has increased considerably, which indicates that previously this method of therapy was not fully employed because of the difficulty in giving fresh blood. When transfusions are made readily available, the clinicians become more aware of possible indications for them. For further discussion see page 468.

RELATIVE USE OF WHOLE BLOOD AND PLASMA

In order to plan properly the operation of a blood bank the relative number of transfusions of whole blood and plasma must be estimated. *The most common indication for transfusion is the treatment of anemia, acute or chronic.* In the therapy of hemorrhagic shock the transfusion of plasma may be of temporary value, particularly when the volume of blood lost is small. In more profound shock, in which greater amounts of blood are lost, the use of plasma can be regarded only as a first aid measure to be employed while the procedures necessary to the transfusion of whole blood are being performed. In chronic anemia the need is, of course, for erythrocytes, and plasma has no value. When patients suffer acute burns plasma is given to replace that lost from the wounds, but even the burned patient frequently develops a degree of anemia which requires the transfusion of whole blood. In any bank the proportion of whole blood to plasma transfusions depends on the types of disease treated and the relative numbers of patients with each type. In many large general hospitals, however, when both whole blood and plasma are readily available in large quantities, transfusions of whole blood constitute from 85 to 90 per cent of the entire number (Table XXVII).

PLANS OF PROCESSING BLOOD

Blood banks are operated to fill many different needs in different institutions. A variety of plans of processing are available to fit these requirements. Care should be taken to select the one which is most suitable and economical for the particular situation.

TABLE XXV

Example of Elapsed Time in Emergencies Between Demand for Blood and Commencement of Injection

Steps in Procurement of Donor	Fresh Blood	Banked Blood
(1) Procurement of a donor	15 min. to 6 hr. (*a*)	——
(2) Determination of the donor's blood group	5 min (*b*)	——
(3) Serologic tests on donor's blood	60 min to 24 hr. (*b*)	——
(4) Determination of recipient's blood group	5 min. (*c*)	5 min (*c*)
(5) Crossmatching of donor's and recipient's blood	5 min. (*d*)	5 min (*d*)
(6) Collection of blood from donor	10 min.	——
Minimum	1 hr. 35 min.	10 min.
Maximum	12 hr. 25 min.	10 min.

Legend:
(*a*) The time can be estimated only approximately
(*b*) The test can be performed *before* the emergency if a donors' registry is employed
(*c*) Rh typing is omitted in emergencies
(*d*) Crossmatching for Rh antibodies is omitted in emergencies.

of the patient may be crossmatched with blood in the bank before operation. The proper flask of blood is reserved for that patient but is kept in the refrigerator, during and after operation, so that it may be administered immediately if required and no time will be lost in performing tests for grouping and crossmatching. Should transfusion prove unnecessary, the flask of blood reverts to the common stock after a specified period, such as twenty-four hours.

Availability of Large Volumes of Blood

The stock in a bank permits prompt transfusion of large volumes of blood in cases of exsanguination. Under such arrangements it occasionally has proved practical to transfuse the patient with severe hemorrhage with the necessary four to six liters of blood in twenty-four hours. Such a problem would be extremely difficult without a blood bank.

Replacement of Blood by Relatives and Friends of Patients

The average patient has a limited number of relatives or friends who can qualify as blood donors and are willing to give blood. If fresh blood transfusions are employed, many potential donors are disqualified because of the blood group to which they belong. When a blood bank is in operation donors of any group may be accepted and their blood exchanged for that of a group suitable for the patient. Thus all potential donors which the patient can muster are utilized to his credit. This consideration is important, particularly if the amount of blood required is large, because many patients cannot afford the cost of blood from professional donors.

ESTIMATION OF NUMBER OF TRANSFUSIONS REQUIRED

In planning a blood bank for a specific hospital the number of transfusions per unit of time must be estimated in arranging for laboratory facilities and personnel. The history of nearly every hospital blood bank demonstrates that the facilities originally provided quickly proved to be inadequate because of failure to anticipate sufficiently the growth in demand for transfusions. There is merit in the policy of permitting a service to grow naturally, but foresight should be employed so that the space allotted for the bank can be enlarged by direct extension.

A number of factors are concerned in such an estimate and the weight of each varies with each institution.

Factors Influencing Number of Transfusions

Efficiency in Operation of the Blood Bank. The clinicians will employ more blood transfusions if the blood bank is operated efficiently and transfusion reactions are reduced to a minimum. Much depends upon the clinicians' estimate of the competence of the staff of the blood transfusion service. If a diligent watch is kept to reduce the incidence of reactions and prompt service is given, more blood will be employed in therapy. The average physician is unfamiliar with the types of reactions to transfusion and the modern advances in the immunology of the blood, but he will delegate the transfusion of his patients to a person in whose ability he has confidence. A careful record of the data on transfusion reactions will assist materially in discussions with the professional staff regarding the incidence and causes.

Types of Disease Treated. Hospitals which receive a large percentage of patients with major traumatic injuries naturally will require facilities for many emergency blood transfusions in the treatment of hemorrhagic shock. In certain industrial areas there may be a high incidence of acute burns so that a disproportionate amount of plasma is necessary. In an institution filled with patients with chronic diseases a considerable demand may be encountered for resuspended red blood cells in the treatment of chronic anemia. Where there are many elective operations performed, there will be many cases in which blood is crossmatched with patients and held pending possible need during or after operation. This results in an increase in the number of laboratory tests in proportion to the transfusions actually given.

Training and Whims of Staff Physicians. In the final analysis, the number of transfusions given in a hospital depends on the capabilities of the clinicians and their knowledge of modern therapy. These factors vary greatly with the training, age, and

Long Storage of Whole Blood. Plasma Made by Sedimentation is the By-product

The maximum use of the erythrocytes is obtained by long storage of whole blood. If the preserved blood becomes outdated, the dilute supernant plasma is aspirated from the sedimented cells and the former is kept for an indeterminate time, to be transfused when required. This plan results in minimal loss of red blood cells. With a normal demand for transfusions, the blood of groups O and A is transfused before the outdating period occurs. More likely to become outdated is the 15 per cent of bloods belonging to groups A and AB for which the demand may not arise before the maximum period of storage expires. Over a long period of time the amount of blood converted to plasma under this arrangement is probably less than 10 per cent (4.1 per cent in Table XXVII). It will be noted that this figure approaches the demand for plasma transfusions in a general hospital.

Short Storage of Whole Blood. Plasma Made by Sedimentation is the By-product

This plan has little to recommend it for any purpose. Usually it is employed because the preservative mixture is incapable of keeping the erythrocytes intact for more than a few days. The number of bloods which attain the outdating period before being transfused is excessive, which results in great waste of red blood cells despite the fact that the greatest need is in the treatment of anemia. With the use of the inefficient preservative mixture considerable hemoglobin frequently appears in the plasma. Centrifugation for the separation of the plasma is unsatisfactory because the erythrocytes have deteriorated to such a degree that many are broken in the process.

Processing of Plasma. Resuspended Erythrocytes are the By-products

Primarily when the production of plasma is desired, the blood is collected in a small volume of an isotonic solution of sodium citrate. The blood mixture then is centrifuged as promptly as possible and the plasma separated. The red blood cells may be resuspended and transfused into patients with chronic anemia. The period of storage of the red blood cells after separation from the plasma is relatively short and many must be discarded unless there is an unusual demand. Erythrocytes must be regarded as a by-product in this plan. There is little to recommend this plan for the hospital blood bank in which the most common problem is the treatment of anemia.

These data are not applicable directly to any institution because of the various local factors which cause deviations from the general picture. Table XXVII contains an estimate of the proportionate use of transfusions by various services in the State University of Iowa Hospitals. It may prove helpful in other situations.

TABLE XXVII

Proportion of Transfusions Given in Various Clinical Services (State University of Iowa Hospitals, 1944–45).*

Clinical Service	Number of Transfusions			Hospital Admissions	Transfusions per 100 Admissions
	Whole Blood	*Plasma*	*Total*		
General Surgery	2185	96	2281	3122	73.0
Pediatrics	354	45	399	1036	38.5
Gynecology	254	2	256	810	31.6
Internal Medicine	540	10	550	2771	19.8
Urology	180	9	189	1686	11.2
Obstetrics	107	4	111	775 women 632 infants	7.8
Otolaryngology	82	1	83	1929	4.3
Orthopedics	125	2	127	3238	3.9
Dermatology	8	0	8	406	1.9
Neurology	15	3	18	1146	1.5
Ophthalmology	0	.0	0	1036	0
Radiology	0	0	0	85	0
Totals	3850	172	4022	18672	All services 21.5
Unclassified (Outside Hospitals	210	14	224		
Grand Totals	4060	186	4246		

* Both whole blood and plasma were readily available in practically unlimited quantities.

STOCK OF BLOOD IN THE BANK

The chief advantage of a blood bank is that blood is readily available at all times. The stock in the bank should be sufficiently large to permit an adequate supply of all four blood groups and a proper proportion of Rh-negative and Rh-positive blood of each group. Theoretically the proportion of blood groups in the bank at any time should be approximately the same as that encountered in the general population; i.e., O, 45 per cent; A, 40 per cent; B, 10 per cent; and AB, 5 per cent; Rh positive, 85 per cent; and Rh negative, 15 per cent. Actually, however, this incidence seldom will be found in stock. The percentage will, of course, hold true in a sufficiently large series and the incidence of blood donors belonging to a certain group will equal the number of recipients of the same

personality of the physician. A doctor may read several articles on the use of plasma and thenceforth order plasma transfusions with little perspective on the problem. Another physician may requisition blood transfusions for tenuous indications or for "desperation therapy." On the contrary, some surgeons have been known to regard the transfusion of their patients, during or after operation, as a personal reflection on their abilities and have reserved the procedure for those who attain only severe degrees of shock or exsanguination.

Many individual opinions may be altered by experience with the operation of an efficient blood bank. Many surgeons have found it feasible to revise upward the maximum age of patients upon which major operations can be performed when a well-functioning blood bank is available.

Increase in Number of Transfusions Because of a Blood Bank

The number of transfusions increases sharply in an institution in which a blood bank is established. This is the result of making blood transfusion less difficult and more available. After the initial increase a steady but gradual increase from year to year may be noted as physicians learn new indications for transfusions or as the general knowledge of preoperative and postoperative care is revised. In Table XXVI are data from the State University of Iowa Hospitals which illustrate this trend.

TABLE XXVI

Increase in Number of Blood Transfusions Coincident with the Operation of a Blood Bank (State University of Iowa Hospitals)

Year	Number of Transfusions	Number of Hospital Admissions	Transfusions per 100 Admissions	Transfusions per Bed per Year
1938–39	552*	7990*	6.9*	1.6
1939–40	2312	19988	11.5	2.8
1940–41	2629	21564	12.1	3.2
1941–42	2830	20996	13.4	3.5
1942–43	3357	19068	17.6	4.1
1943–44	3389	18233	18.5	4.2
1944–45	4022	18672	21.5	5.0

* Data for last five months of year

Proportion of Transfusions in Various Medical Specialties

Some basis for planning the capacity of a blood bank may be obtained by an analysis of the proportionate use of blood transfusions by medical specialists in the various services of a general hospital.

TABLE XXVIII
Disposition of Blood in a Bank (State University of Iowa Hospitals, 1944–45)

Disposition of Blood		Number of Bloods	Per Cent of Total		
TRANSFUSED	Fresh	140	3.2		
	1	170	3.9		
	2	442	10.2		
	3	524	12 1		
	4	489	11.3		
	5	414	9.5	81%	
	6	343	7.9		
	7	271	6.2		
	8	262	6 0		90%
	9	202	4.6		
	10	149	3.4		
	11	118	2 7		
	12	98	2.2		
	13	77	1.7		
	14	75	1.7		
	15	58	1 3		
Days of Storage	16	62	1.4		
	17	52	1.2		
	18	37	0.8		
	19	23	0.5		
	20	12	0 2		
	21	6	0.1		
	22	8	0 1		
	23	4			
	24	4			
	25	1			
	26	1			
	27	6			
	28	2	0.6		
	29	2			
	30	2			
	32	1			
	33	2			
	34	1			
CONVERTED TO PLASMA[1]		180	4.1%		
DISCARDED Positive Wassermann or Kahn Tests		19	0.4%		
Other Reasons[2]		66	1.5%		
Total		4323			

1. Conversion to plasma [illegible] erythrocyte suspensions or plasma, [illegible] smotic fragility of erythrocytes.
[illegible] ge, contamination, clotting, or insufficient quantity to recover plasma.

Waste of Blood in the Bank. The causes of waste of blood in the operation of a blood bank are poor preservation, breakage, blood with positive Wassermann reactions, clotting as a result of improper technique during collection, and outdating of blood during storage. The amount of blood which is discarded because of poor

group. But the number of units kept in stock is not large enough to satisfy the law of probabilities. Furthermore, one or two patients may be present in the hospital simultaneously who require large amounts of blood of the same group and months may pass before the drain on that group is compensated for. For example, a patient of group O required forty flasks of group O blood in ten days for the treatment of continued hemorrhage. This depleted the supply of group O blood in a stock of over one hundred units of miscellaneous groups in the bank. Furthermore, when forty donors replaced the blood which had been used for this patient, it was found that only ten belonged to group O. The result of this chance occurrence disturbed the proportion of the groups in stock for several months.

Optimum Stock in the Bank. Over 80 per cent of the flasks of blood in the bank are used during the first eleven days of storage (Table XXVIII), but many may be held much longer before the need for them arises. In general, the rarer groups, B and AB, remain the longest. If a preservative mixture is employed which permits only a short period of storage, there is an excessive loss of blood from outdating. If too large a stock of blood is carried in the bank, the loss is great, even though a long period of storage is employed. If the stock be too small, many of the advantages of a blood bank are lost.

No exact figures can be given for the optimal stock to be carried in the bank. It is a safe rule to have sufficient blood for a normal requirement of five days. For example, if the average number of transfusions is one hundred per week, seventy flasks should be kept constantly in stock. If fifty transfusions are given weekly, a stock of thirty-five should suffice.

Acquisition of "Capital" for the Bank. If the bank is to be operated on an exchange basis, the stock can be replaced adequately after "capital" originally is acquired. The initial amount of blood in storage when operation is begun should equal the average stock which it is planned to carry. The blood which is used for capital may be purchased from professional donors or given to the bank by members of some charitable organization. It is desirable to acquire the entire capital stock and to begin full operation of the bank within a short time rather than to collect capital gradually. This will minimize the initial loss of blood from outdating.

Before the operation of the bank is begun on full scale, however, it is suggested that one or two "pilot runs" be made in which a few donors are bled, the blood stored, and transfusions finally given. This will test the efficiency of the apparatus and the various steps in the technique. There are always minor corrections to be made which cannot be anticipated.

Relatives and Friends of Patients. Many years before the establishment of blood banks the public was educated to the concept that if patients were to receive transfusions the relatives or friends must furnish the blood. The best source of blood still is the person who is interested in the patient who is to receive transfusion, either because of relationship or friendship Usually they respond well to requests for blood. They frequently combine the donation of blood with a visit to the patient in whom they are interested. The procurement of blood from the relatives and friends of patients can be accomplished by the hospital organization without public appeal for donors. Programs based on this source of blood have the best chance to succeed and require the least effort to contact donors.

Altruistic Persons. There are certain persons in every community who are sufficiently altruistic to give blood for patients whom they have never seen, but, in general, the number is small. Appeals based on unusual need increase the number of persons who give blood. The American Red Cross secured over 13 million blood donations for the Armed Forces during World War II. The number of donors increased during weeks when the war news was unfavorable but notably greater difficulty in securing donors was encountered when victories were reported. Appeals to give blood to children are more successful than when the blood is to be used for adults. The members of hospital guilds, service organizations, churches, police and fire departments, in which the *esprit de corps* is high, are more responsive than the general public to appeals for blood. Members of these and similar organizations can be depended upon to furnish blood in emergencies but the work entailed in securing regular donations from them is proportionately greater. An appeal to the general public is more inefficient and is sustained for long with great difficulty.

Responsibility for Procurement of Donors

The original concept of a blood bank implies some arrangement for the exchange of blood and a method of accounting which involves debiting and crediting. The act of debiting places responsibility for the procurement of blood. A variety of methods are possible by which accounts in a blood bank can be set up and a judicious choice should be made of the one which best fits the local situation. The plan which is employed will contribute importantly to the success or failure of the bank.

Debiting the Patient. When the patient is paying the cost of hospitalization, the most convenient method is to debit his account for the amount of blood which he receives in transfusion. Either the relatives or friends of the patient may replace the blood in the

preservation and outdating can be reduced by selecting a preservative mixture which has a long period of storage. If the mixture has a large volume, the dilute plasma may be recovered from the outdated blood cells. The incidence of positive Wassermann reactions in donors depends, of course, upon the population from which donors are selected. Specific data are given from the experience at the State University of Iowa Hospitals (Table XXVIII).

PROCUREMENT OF BLOOD DONORS

One of the integral features in planning a blood bank is the policy which is chosen for the procurement of blood donors. Frequently the success or failure to obtain sufficient numbers of donors is governed by the approach taken to the problem of blood procurement. To a great extent, the system of records which is used in the bank depends on this.

Sources of Blood

Professional Donors. Since one of the advantages of a blood bank is to make possible the use of blood which is given gratuitously, professional donors should not be in great demand. Professional donors may be employed when patients do not choose to replace blood in the bank but can afford to pay for it. A registry of professional donors should be kept so that physical examinations, blood grouping and typing, and serologic tests for syphilis can be performed before transfusion is required. There are occasional needs for professional donors of a certain group or type when supplies of the proper specificity are not available in the bank. The price paid for blood from professional donors varies in different communities but the average is probably \$5.00 per 100 ml.

Patients. Occasionally patients can be employed as blood donors. Usually this source cannot be expected to provide much blood for the bank. Persons who have disorders not accompanied by infection, deficiencies in blood regeneration, or hypoproteinemia probably can give blood. It must be borne in mind, however, that patients who are to undergo operations or are convalescing require a normal complement of erythrocytes and plasma proteins. Hypoproteinemia, for example, develops after fractures of bones. However, persons with localized diseases of the eye, nose, throat, and some disorders of skeletal system are suitable donors. Blood is suitable for transfusion when it has been collected in the treatment of polycythemia or left-sided cardiac failure. In a properly operated transfusion service it should be unnecessary to depend on patients for any considerable amount of blood.

There are several advantages to this plan. The occasional indigent patient, who has no relatives or friends to replace blood, may be treated from the surplus in the account of the clinical service which assumes responsibility for him. There are relatively few accounts in the bank and consequently only a few persons with which the administration has to deal. The clinical services are compelled, by this system of accounting, to assign their members to specific duties with respect to the procurement of donors. When the administration of a clinical service is inefficient, the procurement of donors is correspondingly poor.

Procurement by Charitable Organizations. In the very nature of the circumstances altruistic persons are best contacted by some group other than the professional staff of the hospital. Charitable organizations may draw entirely on their own membership for blood donors or they may appeal to the general public. Among the organizations which have undertaken such an obligation are churches, Rotary clubs, Kiwanis clubs, Lions clubs, American Legion, hospital guilds, labor unions, and chapters of the Red Cross. The success or failure of the program depends, to a great extent, upon the efficiency of groups completely outside the administration of the hospital. There is the added weakness that this plan depends on altruistic persons as blood donors and their number is dispersed throughout a public which has little interest in the program.

The plan is applicable when donors are sought on a small scale for patients who cannot afford to pay for blood transfusions and have no friends or relatives to replace the blood. The arrangement is necessary in projects in which large numbers of donations are sought for blood to be distributed outside the community where the donors reside.

Appeals to Gratuitous Blood Donors

Various methods of appeal for blood donations must be made, depending upon whether the prospective donor has a personal interest in the recipient of the transfusion. There are relatively few persons who will respond to a general appeal for blood donation. The sense of civic pride may be invoked if the hospital is a municipal project. Persons may be approached as members of a closely knit organization which has offered support for the blood bank. It is the opinion of the present authors, however, that the most successful appeal is on the basis of relationship or friendship with the patient receiving the transfusion. The request frequently is most effective when it is made by the intern or physician who is in immediate charge of the patient. He is best qualified to give information regarding the recipient and the indications for transfusion.

bank or the patient is charged the cost of purchasing blood from professional donors. The funds which accumulate from this source may be employed to purchase blood when it is needed in the bank.

This plan operates through the agency of the business office of the hospital and places the responsibility for the procurement of donors directly upon the family of the patient. The financial transactions may be handled through the usual channels. Of course, this plan is applicable only to cases in which the patient is financially capable of meeting the charges. In the records of the blood bank an individual account is opened for each recipient of transfusion, with suitable debit and credit entries (usually in terms of volumes of blood rather than in monetary values).

Debiting the Physician. This arrangement is applicable to the transfusion of indigent patients. Each physician is given an account in the bank so that whenever he gives transfusions the account is debited; when he procures donors credit is recorded. An important corollary should be that when he has no credit in the bank he cannot give transfusions to patients in his charge. If this is not observed the system fails. The success of such a plan depends on the determined backing of the hospital administration in refusing requests when credit is overdrawn.

The plan is suitable to certain large hospitals in which indigent patients are treated and the immediate care of the patients is largely in the hands of interns and residents. It is particularly appropriate when the clinical services are but loosely organized or there are no divisions based on medical specialties. The disadvantages of the arrangement are two. The accounts of individual physicians are relatively small and seldom show a surplus, so that other methods must be used to provide transfusions for the patients who have no friends or relatives to provide blood. Also there is a chance that the patients of the uncooperative physician will not receive blood transfusions when they are needed.

Debiting the Clinical Service. In general hospitals in which the house staff is large and organized on the basis of medical specialties, accounts may be kept for each clinical service. When a patient is transfused on a service, the service account is debited and when donors are procured, credit is given. The success of this plan depends on the efficiency of the organization of the clinical services. If they are well organized, with a functioning chain of responsibility and administration from the chief to the intern, the plan operates smoothly. Here, again, the system must have the support of the hospital administration to enforce a refusal to give blood to a clinical service when its account is overdrawn. If overdrafts are not refused, the morale of the organization suffers and eventually the plan breaks down.

pointed out that the administration of the blood is only a minor technical procedure in modern transfusion which offers little difficulty. The anesthetist invariably is occupied in the practice of his specialty in the operating room at the precise moment when the blood grouping, typing and crossmatching, so important to success, are being performed in the laboratory. He is, therefore, at some disadvantage in supervising a blood bank. Furthermore, there is no fundamental relation between the body of knowledge in anesthesiology and blood transfusion. The arrangement only succeeds when the anesthetist is genuinely interested in the subject of blood transfusion.

The direction of a blood transfusion service is not a full-time position in most hospitals, although it should be recognized as a minor medical specialty. The routine operation can be entrusted to a technician who can be relied upon to execute instructions and refer special problems to the director.

TECHNICIANS

The technical procedures in the laboratory may be delegated to technicians, nurses, interns, or residents. The recent introduction of complicated techniques for Rh typing and crossmatching demand considerable skill and experience and should not be entrusted to interns and nurses whose knowledge of the subject is casual.

The training of laboratory personnel is difficult and time-consuming. At present the scarcity of skilled technicians constitutes the most serious obstacle to the establishment of more blood banks. Good workers are either self-taught, trained by the director of a blood bank, or have spent a period of time working in some blood bank which is already established. There are few courses available for laboratory technicians in which adequate training is offered in the operation of blood banks.

The technical duties in a blood bank involve some knowledge of nursing procedure as well as acquaintance with laboratory tests and methods. To select technicians it is necessary either to instruct laboratory workers in nursing techniques or to teach nurses to perform laboratory tests. There are advantages to both types of background. Nurses are already familiar with hospital routine. They are trained to work on irregular schedules of hours which may be required in the operation of a blood bank. They are accustomed to responding promptly to night calls for emergencies. On the contrary, laboratory technicians have had the discipline of general laboratory experience which is difficult to inculcate in a novice.

Number of Technicians Required. Since the problems of blood banks vary with local situations, it is difficult to estimate

When the personal approach is not practical the request may be made by mail. This method has had considerable success at the State University of Iowa Hospitals where it has been practiced for years. A form letter is issued by a secretary who has been given the names and addresses of prospects furnished by the patient or his family. The letter merely states that the patient (his name is inserted) is to receive blood transfusions and that the addressee has been suggested as a possible blood donor. A direct request is made for the donation of blood. The qualifications of blood donors are stated in simple terms, together with the hours during which blood is collected. This type of appeal has been surprisingly successful when directed to the friends and relatives of patients.

Whenever possible, the request for blood donation is made *before* the patient actually has received transfusion. This is practical in many elective transfusions. The device capitalizes on the well-known trait of human nature which is epitomized by the proverb that it is exceedingly difficult to pay for a dead horse. After the recipient has been transfused and is convalescent or dead, the response to an appeal for blood is not nearly so successful.

PERSONNEL OF A BLOOD BANK

The Director

A successful blood bank nearly always is the result of the interest and initiative of some physician on the staff of the hospital who has taken the time and energy to inform himself concerning the multitude of details and problems which attend such an undertaking. Many are self-taught by experience and study of the medical literature. The alternative has been to make informal visits to blood banks already in operation. It is a mistake to suppose that a well-functioning blood bank can be organized merely by employing a technician to operate such a service. A physician who has an intimate knowledge of the hospital organization and the confidence of the staff is a *sine qua non* to deal with the personalities of the various clinicians whose cooperation is necessary. Blood banks are being operated successfully by internists, surgeons, pathologists, bacteriologists, and anesthesiologists.

The director who is a clinican frequently has an advantage in designing and operating a transfusion service because of his closer contact with the clinical needs. There is a tendency in some hospitals to assign the responsibility for the operation of a blood bank to the anesthetist. This apparently is done because that person frequently supervises the injection of blood in the operating room. It should be

procedure of drawing blood occasionally is accompanied by serious, though rarely fatal complications.

CENTRALIZATION OF FACILITIES AND PROCEDURES

The collection, storage, and injection of blood involves many procedures, each of which must be performed properly if the recipient is to suffer no untoward results. In addition, there is a great tendency among clinicians to ascribe every unfavorable symptom which the patient exhibits to blood transfusion when that procedure has been used. To a great extent, therefore, the successful operation of a blood bank depends on an organization in which as many procedures as possible are in the hands of trained personnel, under centralized supervision and control. Great effort should be made to devise an unbroken chain of responsibility for the preparation of apparatus for parenteral therapy, the manufacture of solutions used in the preservation of blood, the collection of blood from donors, laboratory tests to determine compatibility, the storage of blood, and the injection of the blood.

Preparation of Apparatus and Fluids for Transfusion

The presence of pyrogens in the apparatus employed for blood transfusion is reflected directly by the number of reactions for which the blood transfusion service is held responsible. The mechanical efficiency of the transfusion equipment is likewise of vital interest to the personnel of the blood bank. Improperly prepared solutions for the preservation of blood may result in dangerous reactions to the recipients and possible loss of blood in the bank. These are cogent reasons why the preparation of equipment and fluids for parenteral therapy should be centralized under the supervision of the director of the blood transfusion service.

Collection of Blood from Donors

Drawing large quantities of blood to be stored and used for transfusion requires more skill than is generally appreciated. The efficient manipulation of the apparatus, the insertion of large gauge needles into the veins of donors, the collection of blood to minimize clotting, the proper storage of blood after collection, and the proper care of donors with adverse reactions from loss of blood are best performed by a physician who is trained by and responsible to the director of the blood transfusion service. The collection of blood from donors is accomplished most conveniently in a room especially equipped for the purpose, adjacent to the laboratory and the refrigerator.

the number of personnel needed. Some factors which govern the amount of work for technicians are: the number of transfusions; the number of Rh typings for the maternity service; the proportion of transfusion procedures assigned to technicians; the number of emergency transfusions performed day and night; the times for collection of blood from donors; the number of records kept in the blood bank; and the amount of plasma processed and the method used for preparation. In the State University of Iowa Hospitals the technical staff consists of a chief technician, three nurse technicians, and a laboratory assistant. This staff is necessary to accomplish about 4000 transfusions per year. In routine transfusions the Rh type of donor and recipient is determined (Rh negative or Rh positive) and crossmatching is performed to detect anti-Rh agglutinins and blocking antibodies. The number of patients admitted to the maternity service is relatively small (Table XXVII). The technicians assist the intern in the collection of blood, perform all laboratory tests, keep the records, and deliver the blood to the recipient in whatever part of the hospital he may be. There are a moderate number of emergency transfusions, day and night, but most occur between the hours of 7 a.m. and 11 p.m. The record system is complex because financial charges are involved in some transfusions. Little plasma is processed and that by the simple sedimentation method. Blood is collected from donors seven days a week, between the hours of 8 a.m. and 4 p.m., and occasionally later. A major portion of the blood donations is made on Sunday. Any deviation from this particular situation in another hospital might require more or less technicians.

House Physicians

Blood transfusion is increasing as a method of therapy and the physician's training should include actual contact with the operation of a blood transfusion service. Two weeks of full-time service in the blood bank by the intern is sufficient to acquire a general knowledge of procedures, a clinical understanding of the types of transfusion reactions, and a superficial acquaintance with the various immunologic tests now employed prior to blood transfusion.

Except in unusual circumstances it is impractical to depend on the rotating intern for the performance of routine laboratory tests because few acquire the experience necessary for reliable results. A service of more than two weeks of the rotating internship probably is unjustifiable. Much better cooperation, however, is obtained when all members of the house staff have had actual service in the operation of the blood bank. The attendance of a physician is desirable when blood is collected from a donor. The

procedure of drawing blood occasionally is accompanied by serious, though rarely fatal complications.

CENTRALIZATION OF FACILITIES AND PROCEDURES

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Custody of the Blood During Storage

The stored blood should be kept in the custody of the blood bank technicians. The flasks should be labeled by them when the blood is collected and the pilots tubes should be identified and attached to the proper flasks by the same person. The blood should be released for transfusion by the same person who performs the compatibility tests. These precautions minimize the number of errors due to mislabeling and mixing of flasks. The refrigerator, where the blood is stored, should be locked in the absence of the technicians and none permitted to enter who is not under the direct supervision of the blood transfusion service. This system will save much confusion and the omission of proper records. Serious errors have occurred from mislabeling when the blood is collected and stored by someone who is not responsible to the director of the service.

Compatibility Tests

It is strongly advised to insist that the laboratory tests for blood grouping, typing, and crossmatching always be performed by trained technicians under the supervision of the blood transfusion service. The modern procedures are complicated and require considerable practice for correct interpretation. The average intern or house officer has not had the experience to perform these tests properly. Accuracy which only can accrue from the discipline of laboratory work should not be underestimated.

Injection of Blood

Ideally, physicians who are responsible to the director of the transfusion service should inject the blood into the recipient. This would insure handling of the stored blood by experienced personnel, from the time of collection until it reached the recipient's vein. If many transfusions are given, however, such an arrangement is impractical. There are many occasions when several transfusions are required simultaneously and too large a staff would be necessary.

A compromise plan has been found more practical. The blood is collected, stored, and tested by personnel of the transfusion service. When a transfusion is required, a properly labeled flask of blood is delivered to the physician in charge of the patient, with assurance that the blood is suitable for transfusion and compatible with the recipient. The patient's physician actually injects the blood. The intern assigned to the transfusion service later visits the recipient to observe signs or symptoms of transfusion reactions. One must guard against certain disadvantages of such

an arrangement. The physician who makes the injection is frequently unskilled in the operation of the equipment he is given to use. Doctors readily admit that practice and skill is required to perform surgical operations but few realize that experience is necessary to perform transfusions successfully. The average physician has little understanding of transfusion reactions and their differential diagnosis and treatment. Many reactions are overlooked or incorrectly diagnosed and treated. Complications also occur from improper handling of blood before injection. Failure to observe precautions as to temperature of the blood, giving the wrong flask of blood to the patient, or the addition to the blood mixture of other substances intended for parenteral administration, all have been observed by the present authors under this arrangement.

PHYSICAL PLANT

If possible the space allocated to the blood bank should be contiguous with that employed in the manufacture of fluids for intravenous injection and the preparation of equipment for parenteral therapy. The entire establishment can be organized as the Division of Parenteral Therapy. The ideal location is one to which the donors find easy access. The site should be selected with due regard to the location of hot and cold water lines, heavy duty electrical outlets, illuminating gas mains, compressed air and steam lines, and telephone cables. The blood bank should be so situated that rapid deliveries of blood can be made to the operating rooms and the parts of the hospital where patients are housed. The plant for the preparation of fluids and equipment is described in Chapter 27.

The blood bank should occupy at least three rooms which are adjacent, a laboratory, a room for collection of blood, and a donors' recovery room. A waiting room for donors and a storeroom are desirable If large scale centrifugation of plasma is performed a separate room for plasma processing should be obtained. Floor plans are discussed in Chapter 25.

LABORATORY

This should be a large room with good natural and artificial illumination. In addition to the equipment necessary for testing blood, space should be provided for office furniture and refrigerators. Outlets are required for electricity, illuminating gas, water, and telephone. A laboratory which is close to the room for the collection of blood is convenient but it should be designed so that

donors will not be required to enter the laboratory to serve as a source of distraction.

Room for Blood Collection

The size of this space should be governed by the number of donors to be handled simultaneously. The arrangements for the time of collection of blood, the number of technicians and physicians who can assist at the same time, and whether the donor tables also are to be used for recovery, all will influence the planning of the room. Good illumination should be furnished. Besides the donor tables it is desirable to have a table or bench upon which to place equipment and flasks of blood. A hopper supplied with hot and cold water will be found convenient in washing apparatus which contain blood clots. Screens protecting the donor tables from view are optional.

Donors' Recovery Room

This room should be adjacent to the blood collection room and easily accessible to it, although the latter should be screened from the view of the occupants. Several large lounges and chairs should constitute the principal furniture. A table upon which to serve refreshments is desirable. Cupboards and a refrigerator for food are convenient. It is particularly desirable to make this room appear as informal and nonprofessional as possible. Suitable equipment should be at hand in case a donor vomits after blood donation.

Donors' Waiting Room

If it is necessary that a number of persons wait simultaneously to give blood, the proper type of waiting room is important. Many prospective donors may not have given blood previously and naturally are apprehensive. The furniture should consist of comfortable chairs and a table upon which are picture magazines. For further reassurance photographs and legends on the walls may depict the various steps in the transfusion of blood.

EQUIPMENT

Refrigerators

There are many refrigerators manufactured for commercial use which are suitable for the storage of blood. Adjustments should be attached so that the temperature can be maintained between 2 and 10° C. Most of the refrigerators of 6 and 8 cu. ft. capacity for domestic use are capable of such regulation without special attach-

ments. The size of the refrigerator will, of course, depend upon the amount of blood to be kept in stock. The cabinet should be vertical so that shelves may be used for storage. The walk-in type is satisfactory for larger blood banks. The apparatus should be so designed that defrosting is continuous to obviate interruptions in service. An alternative arrangement is to have two refrigerators, each of which is large enough to accommodate the entire stock of blood. During most of the time both may be employed for storage but the stock may be consolidated in one when the other is being defrosted or serviced. The refrigerators should be located in or adjacent to the laboratory.

Recording Thermometer. It is extremely useful to install in the refrigerator a clock device with paper upon which is inscribed a continuous record of the temperature. It is important to realize that such a device records the *temperature of the ambient air* in the enclosed space and not the temperature of the blood mixtures in the flasks. Sharp transient variations in the temperature of the air may occur when the door is opened for a short time but the temperature of a glass enclosed fluid changes very slowly and is not much affected by such fluctuations.

Temperature Alarm. Suitable equipment should be installed so that an alarm signal is activated in the laboratory and in the office of the hospital engineer when the temperature in the refrigerator deviates too far from the optimum.

Reserve Refrigeration Facilities. Arrangements should be planned for substitute refrigeration when the regular equipment is undergoing repairs. One plan is the use of two identical units, as mentioned previously. In case the electric power fails completely temporary refrigeration can be maintained by placing ice in the cabinets or by immersing the flasks of blood in ice water. In the latter instance the data on the gummed labels should be transferred to shipping tags and written with a lead pencil.

Arrangement of Shelves. If the stock of blood in the bank is large, it is convenient to arrange the flasks on separate shelves of the refrigerator, classified according to possible uses or stages in processing. A shelf each can be reserved for group A and group O blood and another can be divided for groups B and AB. A separate shelf can hold blood pending reports of serologic tests for syphilis, grouping, and typing. A fifth shelf should be devoted to flasks of blood which are *on call*, that is, blood which has been crossmatched and earmarked for specific recipients and is being held until needed. Other places may be found necessary for outdated blood, liquid plasma, and blood with positive Wassermann reaction.

Facilities for Freezing. In selecting a refrigerator it is well to

consider the desirability of a compartment in which sera of various kinds may be stored in the frozen state. This is a great convenience in keeping grouping and typing sera and specimens of sera to be used for reference. In lieu of such a compartment, a small-sized domestic deep-freeze unit or an ice cream storage device is satisfactory.

CENTRIFUGES

The microcentrifuges recommended for blood grouping and crossmatching are adequate for the procedures ordinarily carried out in the laboratory of a blood bank. A large size centrifuge is required only if plasma is to be processed by centrifugation. This apparatus should accommodate four standard cylindrical flasks of 600 ml. capacity. It should be capable of a speed of at least 4000 revolutions per minute, when loaded. The instrument is preferably mounted in a separate room in which the processing of plasma is accomplished. The 600 ml. cups may be employed for 300 ml. bottles by the insertion of aluminum adapters made by the manufacturer.

DONOR TABLES

These may be adapted from office examining tables, treatment tables, or stretchers, or they may be constructed by any carpenter. The height should be such that the supine donor is brought to a level where working on his extended arm is facilitated. A design which has proved satisfactory with long experience may be constructed and varnished in a general carpenter shop at small cost. The table may be placed beside a wall, permitting the donor's head to be placed at either end, depending on which arm is selected for venipuncture. A slot at either end provides for the insertion of arm rests. An upholstered pad, encased in washable imitation leather, covers the length of the table with the exception of about 24 inches. It is shifted toward the head of the donor so that his feet rest on the bare table. A pillow is placed under the donor's head

AGITATOR FOR BLOOD COLLECTION

Various types of agitators have been devised to mix the blood with the preservative solution in the flask during collection. Shaking is quite necessary when the blood is collected by gravity, whereas when vacuum bottles are employed inversion of the bottle during collection is sufficient. Agitators can be operated by hand, by foot, or by motor and should be designed to accommodate the style of collecting bottle in use. None are commercially available so they must be constructed according to the design of the user,

SPHYGMOMANOMETERS

The aneroid type of sphygmomanometer is particularly useful in the collection of blood from donors. The cuff is used as a tourniquet through which sufficient pressure is exerted on the arm to occlude the veins but not the arteries. When used thus it is found to be quite comfortable.

CABINETS FOR SUPPLIES

Enameled steel cabinets with tight doors have been found suitable for the storage of linen, transfusion equipment (donor), laboratory supplies, and stationery.

OFFICE FURNITURE

The number of records necessary to the operation of a blood bank is quite formidable. An office desk is desirable and it should be complemented with steel files and record books. A typewriter is convenient, particularly in making carbon copies of records and cards.

OVENS FOR DRYING AND STERILIZING

For sterilization of some equipment and drying of glassware an oven is a necessity. Standard laboratory ovens which utilize gas or electricity are designed for this purpose. Ovens which are manufactured for domestic use will be found satisfactory and relatively inexpensive. They are coated with heavy enamel and equipped with heat regulators which are accurate enough for the purpose.

EQUIPMENT FOR BLOOD GROUPING

The apparatus necessary for blood grouping, typing, and crossmatching are described in Chapter 9.

RECORDS AND FORMS

Perhaps the most time-consuming duty in the operation of a blood bank is the keeping of records and the filling of forms. No system of records which is satisfactory for a certain bank is entirely applicable to another situation. A detailed discussion of the records kept in the State University of Iowa Hospitals is presented only to illustrate how a certain set of problems has been met.

THE PROBLEM

In the blood transfusion service of the State University of Iowa Hospitals blood is exchanged for both indigent patients and those

who pay for part or all hospital expenses. For several reasons it is more convenient to debit the clinical services for blood given to patients in their charge. If the recipients are indigent, the members of the staff of the service are responsible for contacting donors to replace the blood, but no financial transaction is involved. If the recipients are paying their hospital expenses, they are given the choice of replacing the blood received or the cost of replacement by the blood of professional donors is charged to their accounts. Although the chief accounts kept in the blood bank are concerned with clinical services, memoranda on transfusions of all patients are sent to the business office where monetary charges are made when indicated. This situation imposes upon the blood transfusion service the task of keeping two separate sets of accounts of each transaction in the collection or administration of blood, accounts for clinical services and accounts for individual patients.

DAY BOOK

This is a large well-bound standard business ledger in which the horizontal lines are ruled across both the left and right pages. On both pages are many vertical columns which can be adapted to the headings desired. The left page is devoted entirely to data on the donors and the right page to the facts concerning the transfusion into the recipient. As donors are received the horizontal lines on the left page are filled in consecutively whereas the lines on the right page are filled when the bloods from the particular donors are transfused, discarded, or converted to plasma. Each donation is given a serial number consecutively in a series which runs for one year and then recommences with Number One. The flasks of blood are known throughout storage by the serial number because it is more convenient than using the donor's name.

The donors' page carries the following headings: serial number of blood, blood group and type, name of donor, address of donor, age of donor, name of patient for whom the blood was given, clinical service to which blood is credited, date when the blood was collected, and the preservative mixture into which the blood was drawn.

The recipients' page (the right page) carries columns for: age of the blood when transfused, date of transfusion, blood group and type of recipient, volume of blood given, name of recipient, age of recipient, clinical service giving the transfusion, location of patient in the hospital, hospital record number of the patient, diagnosis, notations as to transfusion reactions. When the blood is discarded, a notation to that effect is made in red ink in the

space usually reserved for data on transfusion. The date and reason for discarding is noted. If the blood is converted to plasma, the fact is recorded in red ink in the space for transfusion data. When finally the plasma is transfused, the data on the recipient are recorded under this notation.

Service Account Book

This is a smaller book of standard design for business purposes. Each page is ruled for three columns of figures on the right side. A section of pages is allotted to each account, the number of pages in each section depending on the proportionate number of transfusions for the service. The sections are set off by marginal tabs labeled: *General Surgery, Internal Medicine, Pediatrics, Obstetrics and Gynecology, Orthopedics, Urology, Otolaryngology, Dermatology,* and *Neurology.* In addition there are special accounts entitled: *Hospital Account, Sanatorium, Mercy Hospital,* and *Special Pools.*

The Account Book is posted daily from the Day Book. When a donor is sent from the department of Surgery and 500 ml. of blood is withdrawn from him, the figure 500 is entered in the account of the Surgery Service in the column entitled *Credit.* In the third column, marked *Balance,* the continuous balance is increased by the amount of 500. When a patient on the Surgery Service receives a transfusion of 500 ml. of blood, the figure 500 is entered in the second column of the account which is designated *Debit.* Likewise the continuous balance is decreased by a similar amount. The same procedure is employed in all the service accounts. When a patient is transferred from one service to another his credit or debit is transferred with him. For example, if he has a credit of 1500 ml. and is transferred from the department of Internal Medicine to the Surgical service, the account of Internal Medicine is debited by 1500 and a like amount is credited to Surgery.

If plasma is given to a patient instead of whole blood, the charge is made for the volume of whole blood from which the plasma was derived. For purposes of computation 250 ml. of plasma is considered to be derived from 500 ml. of whole blood.

When a paying patient receives 500 ml. of blood from the bank and the blood is not replaced, a charge is placed against the patient by the business office. In effect, this represents a sale of blood from the bank. The Hospital Account is therefore debited by 500. This account can only be credited by the purchase of blood from a professional donor. The blood of professional donors is collected only when the stock in the bank is low or a specific type is needed for immediate transfusion. The Hospital Account therefore usually has a negative balance. When blood is collected

from a professional donor to be used in processing into grouping or typing serum, the Hospital Account is credited.

In this system of accounting it will be noted that the sum of the balances of all the service accounts should equal the amount of blood on inventory in the refrigerator plus the balance owed the bank in the Hospital Account. The balance credited to each department is therefore to be considered as an *asset* and not as an actual amount in the refrigerator. Likewise the hospital is liable to the extent of the difference between the sum of the balances in the service departments and the actual inventory. This value should be carried in the hospital accounts as a liability. These should be liquidated yearly by spreading the whole amount over the various service accounts *pro rata.*

A tuberculosis sanatorium and a private hospital in the vicinity are given service from the blood bank on an exchange basis. Each is given an account in the bank which is treated precisely as a service account within the hospital.

There are occasions when donors give blood to be used for certain types of patients who will be treated in the hospital at some future time. At the time of blood collection the identity of the recipients is unknown. For example, the commissioner of the poor of X County contacts a few persons who are willing to give blood for indigent patients from that county. The blood is collected and credited to *X County Pool.* Withdrawals from this account are made under the authorization of the commissioner of the poor. When a patient on the service of Internal Medicine receives blood from X County Pool, that account is debited by the amount of blood given, and a similar amount is credited and then debited from the account of Internal Medicine.

Patient Account Files

It will be noted that the accounts of the clinical services do not tabulate in any systematic manner the number of transfusions which are given to an individual patient or how many donors have given blood to the credit of that patient. To show these data another accounting system was superimposed upon that for the clinical services. A file of cards, 3 by 5 inches in size, is kept. The cards are filed according to the patients' names in sections marked by index cards which divide the alphabet into approximately 1000 subdivisions. A separate card is filed for each donation of blood for the patient and for each transfusion which he receives. With approximately 4000 transfusions per year, it is estimated that an average of eight cards would be added to any subdivision annually.

Printed forms, 3 by 5 inches, are made up in pads. One form is labeled *Credit* and is printed on white stock, the other is marked *Debit* and is put on yellow stock. Each pad is made up of triplicate forms, the top slip intended for the Business Office, the second slip for the Clinical Service, and the bottom one is a card for the Blood Transfusion Service. The forms are filled out in triplicate and the card is filed in the Patient Account described previously. The text of the forms is as follows: "Credit .... ml. o ..blood to (Name of Patient) (Hospital Number) (Pay Classification) (Clinical Service) (Date) (Pool, if any)." The Debit form is identical except for the word *Debit* instead of *Credit*. Each triplicate combination is numbered serially at the print shop so that the business office may check up any missing numbers which are not received. The forms are posted daily from the Day Book.

In this manner a memorandum of each donation and each transfusion of blood is sent to the business office, the clinical service concerned, and a copy is kept in the records of the blood bank. In the card file the debit and credit records are sorted easily by color of the cards. On the first transfusion card for a patient a note is made of the blood group and type. This frequently is referred to in subsequent transfusions.

Weekly Statement of Accounts

Anyone can receive a verbal statement of the continuous balance of the clinical service at any time by reference to the Account Book. In addition, once weekly a formal statement of accounts is sent to the members of clinical services and the hospital administration. This is made up every Friday morning and gives the status of the accounts at 8:00 a.m. of that day. After the name of each clinical service is given: the number of donors sent in for the preceding week, the amount of blood in milliliters credited to the account for that week, the number of transfusions from that account, the amount of blood debited, and the balance. The figures are totaled for the entire blood transfusion service. Not itemized are the credits and debits which represent transferals because of the complexities in exposition.

Professional Donor Register

For each donor registered as a professional a printed card is kept. This contains the blood group and type, the name of the donor, his address, telephone number, status in the Hospital or University (if any), all typewritten on the bottom. The body of the card consists of horizontal lines with vertical columns entitled *Date. Remarks*, *Volume of Blood Given*, and *Wassermann Report*. These

cards are arranged in a Kardex file of five leaves, each accommodating about fifty cards. One leaf contains the cards of donors of ORh-positive blood, another of ARh-positive blood, a third of BRh-positive and ABRh-positive blood, a fourth of Rh-negative blood of all groups, and a fifth of inactive donors.

When a donor having ARh-positive blood is required, the proper leaf of the file is inspected from the top downward. The cards of the donors who have given blood recently will have red signals on their cards and they are passed over. Those who have been called in order but have not responded will have cards bearing green signals. The top one with a green signal is called again. This process continues until a donor is secured. After donation the green signal is exchanged for a red one. If a donor with a green signal is again called but does not respond, a second green signal is placed on his card. After the third call without response the card is placed in the inactive file to await an explanation by the donor for failure to respond. When no response has been obtained from the donors with green signals, those with no signals are contacted until a donor is secured. At any time the leaf of cards shows a section in which most of the cards have red signals, interspersed with green ones. This indicates the head of the list of donors to be called. Every week the red signaled cards are checked. If three months has elapsed since the donor gave 500 ml. of blood, the red signal is removed. Donors are encouraged to request transfer to the inactive list when they are ill or out of contact for some time.

In addition to the manipulation of the signals, appropriate notations are made on the cards when the donors are called and do or do not give blood.

Requisition for Transfusion and Donors' Record

Any therapeutic procedure which places the patient in jeopardy of his life deserves a detailed account in the patient's clinical record. This principle is recognized in the case of surgical operations; it should be observed for blood transfusion in which the mortality is as high as for some surgical procedures.

The *Requisition for Blood Transfusion* is a form printed on a pink sheet, 8½ by 11 inches, which is designed ultimately to be included in the permanent clinical record of the recipient. The top third of the page contains the data pertinent to the recipient, originally the middle third is blank, and the lower third contains data on the transfusion and untoward reactions.

On the upper part of the page are blanks for the following data: name of recipient, age, hospital number, pay classification, ward,

clinical service, date, diagnosis, history of previous transfusion reactions, stillbirths, or icterus neonatorum, history of hay fever, asthma, eczema, hives, or jaundice. The volume of blood required is stated in milliliters. The time when the transfusion is requested is indicated by encircling one of the following six categories: *Emergency with Rh Typing, Emergency without Rh Typing, Morning Operation, Afternoon Operation, Morning Routine*, or *Afternoon Routine.* Printed explanations are included of the deadlines for each category and the times when blood is available with each.

The requisition is signed by the intern in charge of the patient. A blank is provided for countersigning by the resident on the service who is responsible for securing donors. He may require his approval when he finds too many transfusions being ordered without proper attempts being made to secure donors for replacement of blood.

A boxed portion on the page is reserved for data from the blood transfusion service. In this are recorded the blood group and type of the recipient, the initials of the technician performing the tests, and the date when the tests were made.

When the transfusion is given the record of the donor from whom the blood was derived is stapled to the midportion of the sheet.

The bottom third of the sheet contains the information on the transfusion: the date, the time when the injection was begun and terminated, the urinary findings in the posttransfusion specimen, the various symptoms of transfusion reactions, and the name of the physician who filled out that portion of the form.

The *Donor Record* is a pink slip, 8½ by 4 inches, designed to fit the blank space on the *Requisition for Transfusion*. There are spaces for the donor's name, age, sex, address, and the name of the patient for whom the blood was given. The clinical service to which the blood is credited is noted. There is an assertion that the donor is in good health. The following features in the history are printed, to be encircled: jaundice, urticaria, hay fever, asthma, eczema, malaria, gonorrhea, and syphilis. A question should be included as to whether the donor has ever resided in a malarious country and whether antimalarial drugs were taken. The form is signed by the physician who sends the donor to the blood bank. In the corner of the slip is a box for the serial number of the blood. A larger box is reserved for data of the blood transfusion service: the date of collection of the blood, the volume of blood collected, the preservative mixture employed, the blood group and type of the donor, the date on which the tests were performed and the initials of the person who made them, and the report of the serologic reactions for syphilis.

cards are arranged in a Kardex file of five leaves, each accommodating about fifty cards. One leaf contains the cards of donors of ORh-positive blood, another of ARh-positive blood, a third of BRh-positive and ABRh-positive blood, a fourth of Rh-negative blood of all groups, and a fifth of inactive donors.

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On the upper part of the page are blanks for the following data: name of recipient, age, hospital number, pay classification, ward,

requested). It is signed by the director of the blood transfusion service.

Donor's Waiver

Before blood is withdrawn from a donor he is required to sign a printed form carrying the date and a statement that he is giving blood voluntarily and assumes all responsibility for any untoward results incurred in the donation of blood. His signature is witnessed by one of the personnel in the blood bank. The legal status of this type of waiver is doubtful but it is customary to require it in many blood banks. A few donors remonstrate at signing such a statement but it is probably not wise to accept those who refuse because they may be potential sources of trouble in any event.

Notification Concerning Positive Wassermann Tests

When the Wassermann or Kahn test on the blood of a donor is reported as positive or equivocal, it is felt that the hospital has some responsibility to the donor for this knowledge. On the other hand, not sufficient data are available to make or exclude a diagnosis of syphilis. A form letter is therefore sent to the donor stating that when he gave blood to the bank the results of his blood tests were equivocal and he is advised to consult his physician for an examination and further blood tests. Not uncommonly this procedure demonstrates that the original test was a false positive reaction. Some criticism has occasionally resulted from the physician but this appears to be the best solution to the problem.

Posttransfusion Observation Tags

The intern assigned to the transfusion service makes rounds on the patients who have received transfusions and examines the posttransfusion urine specimens for volume and the presence of hemoglobin. To assist in this task, shipping tags have been printed with the words *Posttransfusion Observation* in red letters, together with a blank for the patient's name. At the time of transfusion one tag is tied to the foot of the patient's bed and the other is affixed to the neck of a urine specimen bottle. The tag on the bed reminds the nurses to collect the urine specimen in the tagged bottle and enables the intern to locate easily the beds of the patients in whom he is interested. The tagged bottles form an easy means of identification when they are placed with other specimens.

Logs for Servicing Equipment

It will be found extremely useful to keep separate logs for each piece of equipment which requires periodic servicing, such as

The *Donor Record* is filled out by the physician who contacts the donor and the latter carries the slip to the blood bank. The data are copied in the Day Book from which the serial number of the blood is obtained and placed on the *Donor Record* slip. After the blood is collected the boxed blanks are filled out. The slips are kept in the laboratory on a clip until the blood is delivered to the recipient or is discarded. The sheaf of Donor Records serves as an inventory of all the flasks of blood in the refrigerator. When the *Requisition for Transfusion* is received and filled, the data from that form are copied in the appropriate places in the Day Book.

Labels on the Blood Flask

Before the blood is collected, the flask containing the preservative mixture carries a label stating the composition of the solution and the date when it was prepared. When the blood is collected into the flask a second label is affixed. This is printed on white gummed paper and bears the name of the donor, the serial number of the blood, the volume of the blood, the date of collection, the preservative mixture employed, the report of the Wassermann and Kahn reactions, and the blood group and type of the donor. When the blood is delivered to the ward for transfusion, a distinctive yellow label is placed on the flask. This bears the statement that the blood in the flask is compatible with that of the patient (name and hospital number given). Two cautions are printed in red ink on the label: "Do not heat the contents of this flask before transfusion." and "Do not add any medication or solution to the contents of this flask, except isotonic saline solution."

Requisition for Determination of Isohemagglutinogens and Isohemagglutinins

This form is sent in with blood specimens when special tests are desired. It is employed particularly for requests for Rh and Hr typing, the determination of anti-Rh and anti-Hr agglutinins, and the titration of cold hemagglutinins. There are blanks for the name of the patient, the age, sex, and date. A statement of the problem is requested. A detailed history of previous blood transfusions is required, giving the dates and whether reactions occurred. A history of each pregnancy is requested with the date, the result (whether full term normal baby, miscarriage, jaundiced infant, etc.). The blank is signed by the physician and his address is given.

The lower half of the form is devoted to the laboratory report, giving the date, the blood group and type, the results of tests for anti-Rh and anti-Hr antibodies, and cold agglutinins (when

Categories	*Deadline for Ordering*	*Blood Available*
Morning Routine	9:00 a. m.	10 a. m. to 5 p. m.
Afternoon Routine	11:00 a. m.	1 p. m. to 5 p. m.
Morning Operation	9:00 p. m. preceding evening	7 a. m. for 24 hours
Afternoon Operation	11:00 a. m. day of operation	1 p. m. for 24 hours
Emergency With Rh Typing	Day or night	60 minutes after order
Emergency Without Rh Typing	Day or night	15 minutes after order

SCHEDULE OF DUTY FOR TECHNICIANS

With the categories of transfusions given in the preceding section the routine laboratory runs of blood grouping, typing and crossmatching are possible at the following times: 9:00 a.m. (*Morning Routine*), 11:00 a.m. (*Afternoon Routine, Afternoon Operation*), and 9:00 p.m. (*Morning Operation*). Emergency transfusions are furnished any time of the day or night. Donors are bled between the hours of 9:00 a.m. and 5:00 p.m. on any day of the week, although occasionally they are received at other hours. The great majority appear on Sunday afternoons when the administration of transfusions is at a minimum.

All four technicians have three half-days off duty every week. They alternate being on call nights, from 11:00 p.m. until 7:00 a.m. There are at least two on duty every morning. One comes on duty at 7:00 a.m. to answer calls from the operating rooms for the morning operations. Another technician is on duty from 3:00 p.m. to 11:00 p.m. on all nights except Saturday and Sunday. It has been found that most night calls occur before 11:00 p.m. and a technician on duty in the early evening minimizes the number of calls made on those who are available for duty later. The evening technician groups and crossmatches blood for the morning operations and also posts the records for the day.

centrifuges and refrigerators. Each log can consist of sheets of paper attached to a clipboard hung near the equipment to which it pertains. The date and type of service should be recorded on every occasion.

Book of Procedures

In each blood bank should be kept a loose-leaf book of procedures containing detailed explanations of all laboratory procedures, the system of keeping records, and daily, weekly, and monthly check lists of duties to be performed, inspections to be made, and reports to be filed. This will aid materially when new technicians are being trained and much confusion will be avoided in the understanding of orders.

HOURS OF SERVICE OF THE BLOOD BANK

The advantages of a blood bank cannot be realized to the fullest extent unless stored blood is made available for transfusion twenty-four hours a day. The completeness of the service depends largely on whether arrangements can be made which will permit technicians to be on call in the blood transfusion service every night.

If the daily number of blood transfusions is small, no special restrictions are necessary as to the time when requisitions should be made. Each blood bank has unique problems in this regard. When the number of transfusions approaches fifteen to twenty per day, the time of the technicians is used inefficiently unless some plan is adopted to permit many tests for blood grouping, typing, and crossmatching to be run simultaneously. This is necessitated with the adoption of the newer methods for typing and crossmatching in which incubation of the serum-cell mixtures for thirty to sixty minutes is required.

A satisfactory arrangement has been evolved at the State University of Iowa Hospitals by which the physician who requests a transfusion indicates the category into which the request falls. The deadlines for ordering and the time during which the blood is available for transfusion are printed on the requisition form. When the blood is crossmatched with that of the recipient it is held in the bank during the time available until a request is made by telephone when it is promptly delivered to the patient for transfusion by the physician in charge of the recipient. If the blood is not called for during the hours in which it is stated to be available, it reverts to the general stock in the bank. The various categories are:

technical direction of the National Research Council and the medical departments of the Army and the Navy. In the meantime, interest in community, regional, and state programs grew and blood services were developed in a number of places in this country during the war. They were operated by such agencies as hospitals, medical societies, and health departments. In some of these either the Red Cross or other voluntary agencies participated in such ways as assisting financially in establishing the service, providing voluntary nontechnical personnel, and recruiting donors for the operating agency.

After the War the Red Cross announced the continuance of the Blood Donor Service to meet the peace-time needs of the country. Chapters were permitted to participate in the nontechnical aspects of programs which served a community, region, or a state.[3,14] These services were to be operated by a recognized medical organization with the Red Cross chapters participating in and financing the nontechnical aspects of donor recruitment and enrollment. The blood and blood derivatives were to be administered without charge for the product. Under this policy Red Cross chapters began to participate in some of the programs already established and in several of the new blood services which were organized from 1945 to 1947. In June 1947 the Red Cross announced a new program designed to provide blood and blood derivatives for medical use on a national scale, without charge for the products, and in sufficient quantities to meet all needs throughout the country.[3] The Red Cross today is prepared to develop, finance, and conduct such a program wherever it is requested to do so.

The Canadian Red Cross Society has also developed a plan for a national blood transfusion service which is being carried out in cooperation with the dominion and provincial departments of health, the medical profession, and the hospitals.[4] The Red Cross Societies in several other countries have also manifested interest in such programs and a few have already developed blood transfusion services.

PUBLIC SERVICE ASPECTS

Janeway[15] has stated: "The sheer number of transfusions given annually is enough to command attention from public health authorities, but there are more compelling reasons: (1) protection of the public from disease transmitted by blood and its derivatives; (2) provision to the public of blood products for the prevention of communicable disease; (3) distribution of blood and blood products for use in the treatment of disease. These reasons indicate an expanding conception of the functions of a health department. The

CHAPTER 25

Community, Regional, and State Blood Services

By John B. Alsever

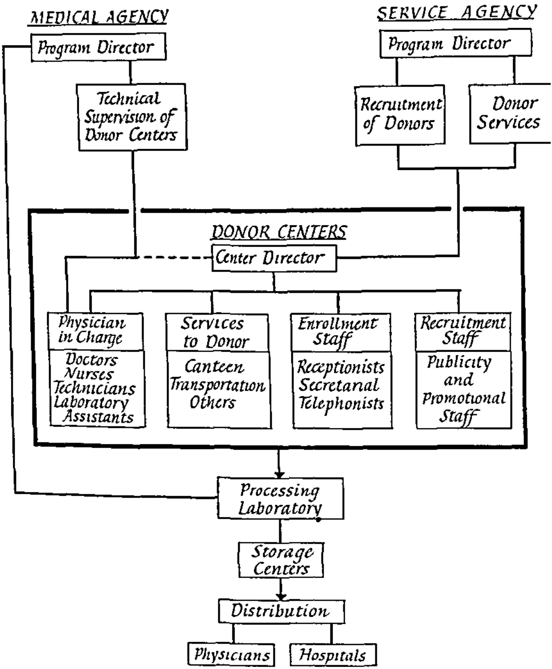
The British Red Cross Society began the organization of donor panels to meet community needs in London in 1921 by having a call list of available donors. This activity grew slowly and in 1925 its value was so apparent that a committee representing the hospitals, the medical profession, the donors, and the British Red Cross Society was established to operate the London service. Similar organizations were also set up in other large cities of England. In 1938 the London service was furnishing about 6000 pints of blood a year. The first organized transfusion service, in which donors were recruited from the general population and bled in advance to supply blood for use in widely separated medical installations, was developed during the Spanish Civil War which began in 1936. In 1939 the British Ministry of Health requested the Medical Research Council to establish a national service to supply whole blood and plasma as a measure for defense. A number of civilian centers were put into operation and the British Army Transfusion Service was organized. These functioned in close cooperation during World War II and the British have since continued the program under the auspices of the British Red Cross Society.[1,2,6]

The policy of the American National Red Cross also embraced programs making blood donors readily available for transfusion. Beginning in 1937 donor panels were developed as a community service in several chapters.[3] Little progress was made in developing widespread blood programs until the entry of the United States into World War II. The Red Cross Blood Donor Service[5] was then organized at the request of the armed services and operated under the

PROCESSING LABORATORY

The requirements of the processing laboratory depend on both the magnitude of the program and the products to be furnished.

ORGANIZATION OF A COMMUNITY BLOOD PROGRAM



Whole blood can be processed for distribution in the donor center or delivered to a central laboratory for processing and distribution. The circumstances of the individual service determine the prefer-

first acknowledges its customary responsibility for the protection of the public from transmissible disease whenever there are accepted methods of control. The second, the provision of agents for the control of transmissible diseases, is a logical extension of the first. The third, however, represents an excursion of public health agencies into the practice of medicine and therefore demands justification." Janeway goes on to point out that this justification appears to be in the social and economic spheres, since blood and its derivatives are now essential to good medical care and, unless they are distributed under public auspices, the cost will deprive many patients of their benefits unless the present costs can be quite considerably reduced.

ORGANIZATION OF COMMUNITY PROGRAMS

The type of organization required for a community, regional, or state blood program includes: (1) adequate medical control and supervision of the technical aspects of the program; (2) a properly equipped and staffed processing laboratory; (3) donor centers of adequate size and suitable location to provide convenient access for the donor population; (4) an organization to recruit and enroll a sufficient number of volunteer blood donors and to render the necessary adjunct services such as transportation and canteen service; and (5) facilities to store and distribute properly the blood and blood derivatives to physicians and hospitals as they are required for clinical use.

On page 499 is a diagram of the type of organization which is employed in many programs being operated in this country, patterned after the experience gained in the American Red Cross Blood Donor Service of World War II.

MEDICAL AGENCY

It is essential that an organized medical group control and supervise the technical aspects of a blood service. The size of the program governs the number and type of personnel required, and whether full time or part time. The medical agency may be organized within the health department, the medical society, or the hospital association in the area to be served, or it can be an entirely separate agency representing these groups. A state program requires a number of full-time personnel, professional, technical, and lay, for adequate direction and supervision, while a program serving a moderate sized community may require only the part-time services of one physician, plus a few nurses and technicians who may also be employed part time.

Equipment. The center should be well equipped and attractive. Among the important matters to which attention should be given are: (1) reading tables, and comfortable chairs in the waiting room; (2) the necessary desks, cots, and bleeding tables; (3) good lighting throughout; (4) adequate toilet, dressing room, coat room and canteen facilities; (5) music and a tastefully decorated interior; and (6) adequate refrigeration, storage space and equipment for cleaning, washing, and sterilizing technical apparatus.

TABLE XXIX

Donor Center Personnel For 160 Donors Per Day

Center Director
Secretary (⅓ time)

NONTECHNICAL STAFF	*TECHNICAL STAFF*
Recruitment	Physician in charge
Director	Secretary (⅓ time)
Assistant (1)	Medical Secretary (1)
Secretary (1)	Head Nurse (1)
Staff (number depends on amount of contact work)	Nurses (2)
Enrollment	Nurses or technicians (6)
Receptionists (4)	Laboratory Assistants or Technicians (2)
Secretaries (4)	
Telephonists (4)	
Services	
Supervisor	
Assistant (1)	
Secretary (⅓ time)	
Canteen (4 to 6)	
Transportation (2 or 3 drivers, each with automobile)	
Others (nurse's aides, attendants, etc , 8 to 12)	

Staff and Functions. The approximate number of staff members required for a center in which about 160 donors are bled daily is summarized in Table XXIX. Experience has shown that many of these positions can be filled by volunteers, especially if a civic agency such as the Red Cross is participating in the program. The number and type of personnel required and their functions are described briefly in the above table.

Reception of Donors. Four receptionists (*a*) greet the donors and (*b*) escort them to the coat room, to the registration desk, and to the nurses taking the medical history.

Registration. Four secretaries (*a*) check the donor's appointment, and (*b*) write the nonmedical data on the donor record card.

able method. Blood plasma may be prepared under a similar arrangement, although centralization is probably desirable for the preparation of plasma and whole blood when there are several donor centers in a population area. In a regional or state program centralized preparation of plasma is dictated by economy. If plasma fractions are desired, their processing must be centralized for efficiency and economy. The commercial laboratories can prepare plasma and plasma fractions on a cost plus contract, receiving blood or plasma from the collection laboratory and returning the finished products. It is generally agreed, from the experience gained during World War II, that only a few of the most populous states in the country can economically establish and operate plasma fractionation laboratories.

DONOR CENTERS

The experience in the donor centers operated by the American Red Cross for the armed forces from 1941 through 1945 has yielded considerable information concerning the most convenient space arrangement, the staffing, and operation desirable for community donor centers.

Fixed Centers

The establishment of a permanent donor center is desirable in densely populated areas where the requirements for blood necessitate frequent or daily bleeding.

Floor Plans. Examples of floor plans for a fixed center which will accommodate twenty donors per hour, or 160 per eight hour day are shown on page 502. These were prepared under the supervision of the author while he was associated with the American Red Cross. The plans do not include space for the processing of blood. Additional office space may also be desirable, particularly for files and publicity activities, in the two one-floor plans. Some features are worth comment: (1) a separate entrance and exit avoids the contact of prospective donors with those who have developed untoward reactions; (2) the double entrance to the recovery room permits the donor to pass from the bleeding room to the recovery room and thence to the canteen; there is also easy access from the canteen to the recovery room in the event of delayed reactions; (3) the location of the coat room facilitates the storage of outer clothing adjacent to the separate entrance and exit during cold or rainy weather; (4) rejected donors can pass directly into the canteen and leave; and (5) other space is located so as to provide the most direct flow of traffic for both the donors and the staff.

Equipment. The center should be well equipped and attractive. Among the important matters to which attention should be given are: (1) reading tables, and comfortable chairs in the waiting room; (2) the necessary desks, cots, and bleeding tables; (3) good lighting throughout; (4) adequate toilet, dressing room, coat room and canteen facilities; (5) music and a tastefully decorated interior; and (6) adequate refrigeration, storage space and equipment for cleaning, washing, and sterilizing technical apparatus.

TABLE XXIX

Donor Center Personnel For 160 Donors Per Day

Center Director
Secretary (½ time)

NONTECHNICAL STAFF	*TECHNICAL STAFF*
Recruitment	Physician in charge
Director	Secretary (½ time)
Assistant (1)	Medical Secretary (1)
Secretary (1)	Head Nurse (1)
Staff (number depends on amount of contact work)	Nurses (2)
Enrollment	Nurses or technicians (6)
Receptionists (4)	Laboratory Assistants or Technicians (2)
Secretaries (4)	
Telephonists (4)	
Services	
Supervisor	
Assistant (1)	
Secretary (½ time)	
Canteen (4 to 6)	
Transportation (2 or 3 drivers, each with automobile)	
Others (nurse's aides, attendants, etc., 8 to 12)	

Staff and Functions. The approximate number of staff members required for a center in which about 160 donors are bled daily is summarized in Table XXIX. Experience has shown that many of these positions can be filled by volunteers, especially if a civic agency such as the Red Cross is participating in the program. The number and type of personnel required and their functions are described briefly in the above table.

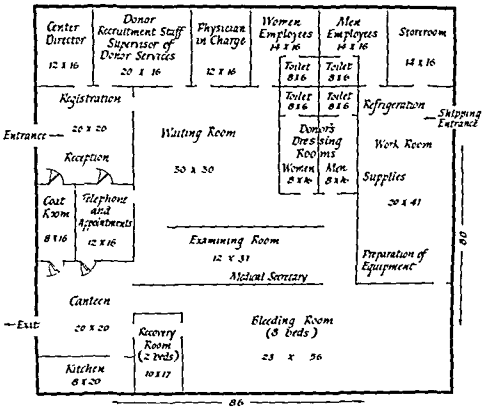
Reception of Donors. Four receptionists (*a*) greet the donors and (*b*) escort them to the coat room, to the registration desk, and to the nurses taking the medical history.

Registration. Four secretaries (*a*) check the donor's appointment, and (*b*) write the nonmedical data on the donor record card.

Appointments. Four telephonists (*a*) receive incoming calls, (*b*) make appointments, and (*c*) contact donors when necessary.

Examination of Donors. (*a*) Two nurses take the medical history and measure the blood pressure (for medicolegal reasons this should be done by a registered nurse or a doctor), (*b*) Two nurses or technicians determine the donor's hemoglobin concentration and the

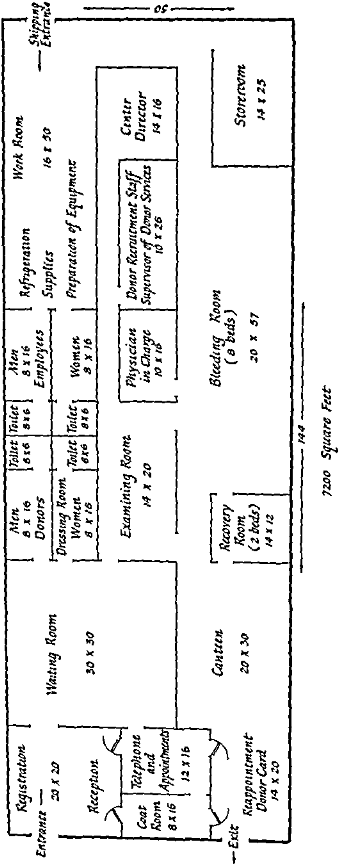
FLOOR PLAN FOR A BLOOD DONOR CENTER (PLAN A)



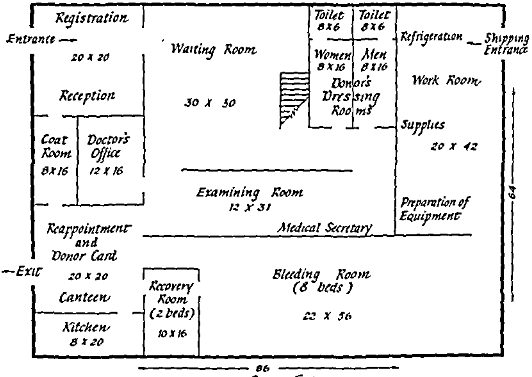
blood group, if the latter is done before bleeding, (*c*) A physician may examine the donor (as a rule, in this type of program, the physician sees the donor only when the nurse's or technician's findings indicate possible rejection, a decision which he makes, (*d*) Two to four nurses' aides or attendants escort donors to and from the waiting room for the medical examination and then to the bleeding room, and deliver the completed donor cards to the bleeding room so that the donors may be called in turn.

Bleeding. The bleeding team consists of (*a*) the physician in charge and the head nurse, (*b*) a medical secretary, (*c*) four nurses or technicians (bleeders), (*d*) four nurses' aides or attendants to assist in the bleeding room, escort donors to the canteen or the recovery room, and to remain with donors in the recovery room, and (*e*) two laboratory assistants or technicians. The work unit is

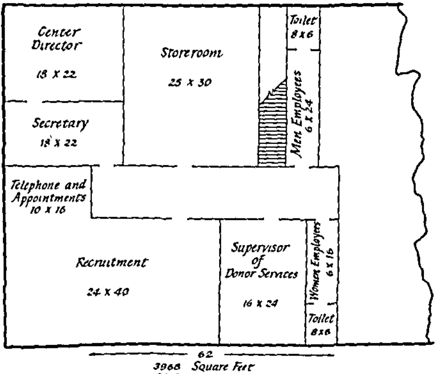
PLAN FOR A BLOOD DONOR CENTER (PLAN B)



FLOOR PLAN FOR A BLOOD DONOR CENTER (PLAN C)



5504 Square Feet
FIRST FLOOR



3968 Square Feet
SECOND FLOOR

equipped with two bleeding tables and a supply table operated by one nurse or technician. The listed personnel can operate eight bleeding tables and handle twenty donors per hour. The composition of the group may be varied to suit the needs of a particular center. For example, when there is more than one bleeding team, there should usually be a physician supervising each team, in addition to the physician in charge of the technical operations of the center. However, one doctor can supervise more than the four bleeders of a team which bleeds twenty donors per hour. Experience has indicated that ten bleeders are probably the maximum number which can be satisfactorily supervised by the doctor. There should also be at least one nurse in each group of bleeders. One medical secretary can adequately handle the donor records for a team of ten bleeders. The records include the donor card, the tag for the blood bottle, the tag for serologic tests and statistical data. The number of finished bleedings per hour should average from four to five per bleeder.

The welfare of the donors, the medical attention they receive, and the technical procedures are entirely the responsibility of the physician in charge. He should be present at all times when donors are being bled. The nurses and technicians carry out the actual bleeding of the donors, except where the doctor's assistance is required in unusual situations. The laboratory assistants or technicians are usually responsible for such duties as: refrigeration of the blood, maintenance (cleaning, preparation and sterilization) of a sufficient supply of bleeding sets, records of the bleedings, inventory of supplies and bleedings, and the care of laundry.

Recovery Room. Under the direct charge of the head nurse and the physician, a room equipped with two beds is ordinarily adequate for a twenty donor per hour schedule.

Canteen Service. Four to six workers can provide food and refreshment approved by the physician in charge, depending on the amount and variety to be prepared and served.

Discharge of Donors. Two or four nurses' aides or attendants can thank the donor, prepare and present the usual donor card, and request a return appointment, arranging for it if the donor so desires.

Transportation. Two or three drivers with automobiles provide transportation which is often essential, and is always desirable, when a donor has had an untoward reaction. The cars may also be useful at times to call for prospective donors and for other business of the center.

Mobile Centers

Whenever the requirement for donors in a given geographic area is not sufficient to justify a permanent or fixed center, the mobile

unit type of operation should be employed. The bleeding team, with its equipment in a truck, visits the area, sets up operations in temporary quarters and conducts a bleeding clinic for one or more days. Adequate space is most important. A gymnasium or hall which provides sufficient free floor space is to be preferred, since it can be easily partitioned off with screens to provide room arrangements similar to those suggested for the fixed center. However, toilets and facilities for washing of equipment must be available and kitchen facilities for canteen service are most desirable. About 1500 sq. ft. (139.35 sq. m.) of floor space is adequate to provide the required working areas for a six to eight bed bleeding unit. The personnel required and method of operation of a mobile center is essentially the same as has been described for the fixed center.

SERVICE AGENCIES

When the blood program is relatively small and operated in one hospital in the area, there may be no need for a distinct service agency. However, the participation of a civic organization in donor recruitment and other nontechnical activities is most helpful. It is certainly desirable, if not essential, in a large program. The recruitment of volunteer donors in sufficient numbers is a task requiring considerable continuing effort. The good will and interest of the general public is necessary. The use of volunteer donors is essential if costs are to be kept reasonable (p. 508), and most people in good health are willing to give blood occasionally if the need for it is clearly and properly presented. The service agency can also contribute greatly in providing and equipping the physical plant of the donor center, in furnishing the nontechnical staff, and in performing nonprofessional duties. As indicated in the introduction to this chapter, The American National Red Cross is prepared through its chapters to provide this type of assistance at its own expense, and, since June 1947, it has offered to finance and carry out the entire program at request, supplying the blood and blood derivatives for use without charge for the product. Many other civic organizations have participated in individual programs throughout the country and doubtless will continue to do so because of the interest of the local groups.

DISTRIBUTION

The blood and blood derivatives must be handled and distributed through facilities which will keep in reserve storage adequate amounts to meet emergencies and to insure the maintenance of

sufficient supplies in hospitals and other depots. Thus the required product will always be on hand when the physician needs it for the treatment of a patient. This may be a logical responsibility of the health department, since it ordinarily has facilities for the distribution of biologics and other products. Distribution may also be undertaken by the service agency as a part of the operation of the donor center, or as a centralized activity serving a larger area in which several centers operate.

BLOOD REQUIREMENTS AND COST ESTIMATES

The rate of use of blood and plasma for transfusions in a community has been suggested by data collected in 1944, based on the experience of a number of large hospitals where well-operated blood banks have made adequate amounts of blood and plasma available at a reasonable cost. In an average general hospital, this has been estimated to be about four transfusions per bed per year, approximately three fourths or more of which should be whole blood. Translated in terms of population figures, this would require one blood donation per year per fifty people. Therefore, one can assume that about 20,000 blood donations per year should approximate the needs of a community of 1,000,000 people. A more accurate determination of such needs must await further experience in the operation of community, regional, and state programs. Current experience in one large hospital would suggest that the quoted figures might be doubled or trebled in the future as the use of blood becomes more convenient.

Cost estimates must also be based on the translation of the available data derived for the most part from other types of operation during 1944 and 1945, since blood programs serving large areas and providing a variety of products have not been conducted for a sufficiently long period as yet. Some of the cost data come from operations of a much greater volume than could exist in anything but a nation-wide program. For example, the average cost per bleeding in the national wartime program of the Red Cross was about $1.00. With the smaller volume in a large state program, this cost might amount to $1.50 per bleeding; while in a smaller community program, with intermittent operation of the center, the cost might well be somewhat greater. The preparation of liquid and dried plasma in two state-wide programs has cost about $2.00 to $3.00 per unit of finished plasma. In a smaller community program, this cost might also be expected to be higher. The experience of one of the commercial laboratories during World War II, where a large fractionation program operated at an

optimum rate of production, suggests that the useful plasma fractions can be manufactured and packaged for a cost of about $5.00 per bleeding. With the smaller volume of any state-wide program, this might well amount to $6.00 per bleeding, or more. This cost estimate for fractionation may or may not be applicable to the optimum operation of a smaller laboratory, since no data are available. In any event, the initial equipment costs are quite high and a specially trained technical staff is required.

On the basis of the quoted costs, one can arrive at an estimate of the operating costs for a program of the type discussed. It is our opinion, since even state-wide programs in most instances will be small as compared with those carried on during the war, that the costs will probably be somewhat higher than the estimates in the preceding paragraph. The total average cost per bleeding has been arbitrarily increased by $1.00 to allow some leeway in this regard. Assuming that a well-balanced program will provide 70 per cent whole blood, 20 per cent plasma and 10 per cent plasma fractions, plus red cell suspensions and derivatives, the costs per bleeding would be about as follows:

(1) *Complete program, including fractionation products:*

70% whole blood at	$1.50 × 7	$10.50
20% plasma at	3.00 × 2	6.00
10% fractions at	6.00 × 1	6.00
		$22 50

The average of $2.25 plus $1.00, results in an estimated average cost per bleeding of $ 3.25.

(2) *Blood, plasma and red cells only:*

75% whole blood at	$1.50 × 7.5	$11.25
25% plasma at	3.00 × 2.5	7 50
		$18.75

The average of $1 88 plus $1.00, results in an estimated average cost per bleeding of $ 2.88.

On this basis, the annual cost of a program for a community of one million people (20,000 bleedings) would be about $65,000 ($0.065 per capita) for the complete program, or about $57,600 ($0.0576 per capita) for blood, plasma, and red cells. These figures are based on the assumptions that: (1) the blood will be provided by volunteer donors; (2) the nontechnical personnel required in the donor center operations will be largely volunteers; (3) if fraction products are provided, the processing will be done as a part of the operation of a laboratory serving many other programs. For example, the common professional donor fee of $5 00 per 100 ml. of blood would increase the estimated cost per bleeding by $25.00; payment of all donor

center personnel would add from $1.00 to $2.00 to the average unit cost per bleeding; and the cost of fractionation on a small scale would be very high.

PROGRAMS IN OPERATION

There are a fairly large number of community, regional, and state programs of various types now in operation. No attempt is made here to describe or even to list all these programs. A few, which have been in operation for some time and represent different types of activity and organization, have been selected for brief description as illustrative examples.

Hospital Blood Bank Serving a Metropolitan Area

Hoxworth[10,11] has developed a program which provides service for hospitals in Cincinnati, Ohio. The donor center and processing laboratory are in the Cincinnati General Hospital and provide whole blood, red cell suspensions, and liquid, frozen, and dried plasma. The staff consists of a medical director, an assistant, a secretary, five technicians, a laboratory assistant, and four part-time volunteers. The program operates day and night and provides service on request to fifteen hospitals in the metropolitan area. Since most of these hospitals maintain their own transfusion services, most requests are for emergency or unusual needs. Blood and plasma are provided on a plan of replacement per unit by any of the following arrangements: (1) one volunteer donor and a $12.50 service charge; (2) a service charge of $25.00; (3) two volunteer donors; (4) no charge to indigent patients, regardless of the donors provided for replacement. The charge for red cell suspensions is one half the rate for whole blood. These replacement requirements are not rigidly enforced but are adjusted to meet the circumstances of the individual patient. Hoxworth reports that this system has always provided an adequate supply of donors to meet the demands on the service. Bleeding clinics are held three half-days a week on an appointment schedule and the physicians on duty are paid by the participating hospitals *pro rata*, an average of $0.40 a donor. The service is provided free to patients in the Cincinnati General Hospital and donors for these patients are accepted at any time. In return, the Hospital furnishes the space for the blood bank without charge. The rate of transfusion more than doubled during the first five years of operation, reaching an annual rate of over 5000. The director serves without pay and the program has been solvent financially. The average cost has been somewhat less than $2.00 per unit.

Hospital Blood Bank Serving Towns in Wide Area

Davenport,[12] in charge of the blood bank at the Southern Baptist Hospital, New Orleans, Louisiana, has developed a somewhat different type of program. Regular service is not desired by most of the other hospitals in the metropolitan area, since they operate their own blood banks. The program specializes in the operation of a mobile unit which services the small nearby communities where the maintenance of a blood bank would not be practical. This includes towns within a range of about 150 miles in Mississippi and Louisiana. The method of operation is cooperative. The town provides (1) the donors, (2) the space for the bleeding clinic, (3) the clinic equipment, canteen service, the necessary nontechnical personnel (ordinarily volunteers), and (4) two or three nurses (or equivalent) to assist in the bleeding and examination of the donors. The mobile unit provides (1) supplies for registration, (2) equipment for bleeding and for refrigeration and transportation of the blood, (3) material for grouping of the donors (a list is left for local use), and (4) the physician in charge. The town receives two fifths of the plasma prepared from the blood obtained, without further cost. Administration sets are furnished with the plasma and replaced as the used sets are returned. Additional plasma is furnished on request with a maximum fee of $10.00 per unit. Whole blood, red cell suspensions and paste are also available. When service is requested in the city of New Orleans, private patients are charged $5.00 per unit, or the blood from one volunteer donor. Charity patients are not charged, but replacement of blood is solicited. Service was provided to forty-eight hospitals in twenty-four communities in 1943.

Independent Nonprofit Blood Bank

The Blood Bank of Dade County, Miami, Florida,[20] is an example of still another type of operation. It is housed in a separate building which is located on the grounds of the city hospital, but owned by the Blood Bank. It is a nonprofit institution operated by a board of directors and originally financed by public donation. It provides service to all of the hospitals in the city of Miami and Dade County. It also serves as a cooperating unit of the regional or district blood bank system which has just been established to serve the entire state of Florida. In May 1947 the district banks were organized as the Florida Association of Blood Bank Services to expand and develop their activities so that adequate service would be assured throughout the entire state. Branch blood banks are being established where geographically desirable. Distribution of blood and blood derivatives is made through hospitals. The state department of health has

agreed to provide a mobile unit so that donor clinics can be held in the smaller communities. Blood bank methods are being standardized for the entire group. A system of exchange has been established so that emergencies can be met and a person may donate blood in one part of the state to replace the blood given to a patient hospitalized in another. The Dade County Blood Bank provides whole blood, cell suspensions, liquid plasma, grouping serum, Rh-typing serum, Rh-antibody studies, and includes a rather well-developed research laboratory. The bank serves thirty-two hospitals. It gave 12,000 units of blood, plasma and cell suspensions in 1946, of which approximately 40 per cent were to charity patients. The donor problem is reported as somewhat difficult. For example, the replacement rate for private cases, including fees, was only 87 per cent from July 1946 through June 1947, while charity patients replaced only about 30 per cent of the blood, plasma and cells given. The city and county pay for part of the cost of the charity service. The use of red cells suspensions wherever this meets the clinical need, plus the availability of surplus Army-Navy plasma, is currently making up for the donor deficit.

Blood Bank of County Medical Society

The county medical society in San Francisco was one of the early groups interested in developing a community blood service. Its method of operation is similar to the Cincinnati program. The society operates the service in quarters which they provide. Blood, plasma (dried or liquid), and cell suspensions are provided on a nonprofit basis to the hospitals of the San Francisco area.

State Department of Health Plasma Program

The Michigan State Department of Health was the first to organize a plasma program on a state-wide basis.[13,17,18,19] The program was developed as a part of the wartime program for civilian defense in 1943. From the beginning, the local chapters of the American Red Cross have participated as the service agency and have provided publicity, volunteer donors, space and furnishings for the mobile unit to conduct its bleeding clinics, the required number of volunteer nontechnical assistants, the canteen, and other related services. The Department of Health provides a mobile unit and all the technical personnel, including the physician in charge, all technical equipment for bleeding, and facilities for refrigeration and transportation of the blood. Visits are scheduled to those communities which request the service. The processing laboratory is housed in the state laboratory buildings at Lansing. Liquid plasma is prepared and distributed throughout the state, using the local

hospitals as supply depots. Although emergency needs are met anywhere, the availability of plasma is in direct proportion to the number of blood donations made by the residents of each community. Plasma is provided without charge for the product. Stocks are replaced in each hospital as needed, provided the community participates in the program. In 1945, 130 hospitals in seventy-five of the eighty-three counties in the state were being supplied as participating groups. About 5000 units of plasma had been distributed during the previous 12 month period and the rate of use of plasma was slightly less than two units per general hospital bed per year. The unit cost, high at first, was estimated to be about $2.00 per bleeding at the end of 1945. The Department of Health plans to add whole blood, cell suspensions, and plasma fractions to the service in the near future.

The North Dakota program,[7,16] which was begun in 1944, is essentially similar to the Michigan service, except that dried instead of liquid plasma is provided and, in the beginning, the State Department of Health arranged with the local civic organization most interested to act as the service agency. The Red Cross chapters entered the program as the official service agency in 1945 at the request of the State Department of Health. At the close of the first full year of operation, the service had supplied plasma to thirty-five of the fifty-three counties in the state, and nearly 1400 units of plasma had been used. The unit cost the first year was $12.56 but it was estimated that it would be less than $3.00 during the second year of operation.

State Department of Health Complete Blood Program

The Massachusetts State Department of Public Health[21] in December 1945 began the first program designed to provide whole blood, plasma, cell suspensions and paste, and plasma fractions. The service is on a state-wide basis. There are mobile unit operations, of the type already described, and by May 1947 153 communities had been visited, with a total donor registration of 19,204. The present plans contemplate semi-independent fixed center operation for the large cities and include participation of hospital blood banks. It is expected that all the products will shortly be made available to some degree. The processing laboratory is part of the department's Biologic Laboratory, located just outside of Boston. This program, like the one in Michigan, has been organized with the Red Cross chapters participating as the service agency.

Other Varieties of Operating Programs

The Illinois State Department of Health[9] supplies plasma to hospitals in the state provided (1) they request to partici ate, and

(2) agree to the regulations of the program. The state agrees to supply one unit per ten hospital beds and the hospital agrees to (1) store it properly, (2) administer it properly, (3) maintain the inventory, and (4) replace that used within one week. Replacement may be by (1) purchase, (2) shipping 500 ml. of acceptable blood to the central laboratory (Samuel Deutsch Serum Center) plus a payment of a service charge of $7.50, or (3) shipping 1000 ml. of acceptable blood to the laboratory.

Arkansas[8] developed a limited program sponsored by the State Medical School and supported by private donation. It has since been extended to include the entire state on the basis of county service organizations and the operation of a mobile unit. Dried plasma is supplied to the local hospitals in a county in amounts equivalent to the blood donated at each visit of the mobile unit. Plans are under way to expand the service to include whole blood and cell suspensions. The charge to the patient is determined by the patient's physician and may range from nothing to $10.00. These fees are used to help defray the expense of the program.

More recently community programs have been developed by Red Cross chapters in several cities with the advice and approval of the medical society and the hospitals. Notable examples are Los Angeles, California, and Detroit, Michigan. The chapter finances the entire program in some instances, in others the health department or other medical group assists. Most other programs have started by providing a limited service, although the plans ordinarily provide for eventually supplying the needs of the entire community, when the service is fully developed.

Comments

The organization of community, regional and state programs is most interesting. It appears to be a sound development, both economically and professionally, from the standpoint of the total effort, equipment, and facilities involved, when compared to the customary method of meeting the community needs for blood and its derivatives by means of individual hospital programs. If community and state operation proves to be as successful as the existing programs indicate, it is likely that this type of program will eventually blanket the country. However, the coverage of too wide an area by one donor center (or mobile unit) is not sound; the supply of whole blood must be under local control to function properly (city, county or small groups of counties); and adequate donor recruitment seems to depend on the development and maintenance of a strong community spirit and sustained interest. The latter cannot be done unless the organization of these programs takes cognizance of the individuality of communities and regions within states and

the necessity for local color in presenting the need for blood donors to the public.

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CHAPTER 26

Transfusion Equipment

By Elmer L. DeGowin

The methods and apparatus for blood transfusion are legion. Despite frequent warnings that the operation should be made as simple as possible, for the last 300 years the transfusion problem has stimulated ingenious and mechanically-minded physicians and others to devise complicated machines which are expensive, difficult to manipulate, and formidable to clean. Many procedures have been based on the premise that citrated blood is harmful so that elaborate means have been designed to transfer blood from donor to recipient without the addition of an anticoagulant. The experience with transfusion since the introduction of sodium citrate as an anticoagulant in 1914 has demonstrated the fallacy of this assumption and therefore the lack of the necessity for many of these devices.

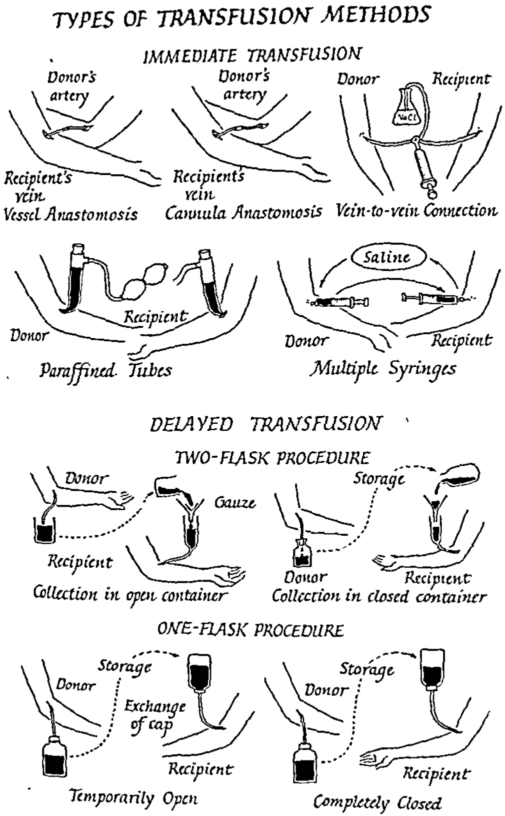
The very multiplicity of transfusion methods is sufficient evidence that none is yet perfect. In the United States, however, there has been a notable trend in the last ten years towards a simplification and standardization of transfusion apparatus. This has resulted from the increased use of stored blood and the rapid expansion of the commercial production and distribution of fluids for parenteral therapy. In general, this has been a beneficial development. However, the apparatus for transfusion, which has had wide distribution, leaves much to be desired. The equipment has been designed primarily for the transfusion of fresh blood, which is notably easier to manage than preserved blood. It is the opinion of these authors that much improvement can still be made in apparatus for the transfusion of preserved blood.

The transfusionist should be cautioned against a hasty decision to employ homemade transfusion apparatus rather than to use that which is readily available commercially. Such a project may prove

the necessity for local color in presenting the need for blood donors to the public.

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TYPES OF TRANSFUSION METHODS
IMMEDIATE TRANSFUSION
Donor's artery
Recipient's vein
Vessel Anastomosis
Donor's artery
Recipient's vein
Cannula Anastomosis
Donor
Recipient
NaCl
Vein-to-vein Connection
Donor
Recipient
Paraffined Tubes
Saline
Donor
Recipient
Multiple Syringes
DELAYED TRANSFUSION
TWO-FLASK PROCEDURE
Donor
Gauze
Recipient
Collection in open container
Storage
Donor
Recipient
Collection in closed container
ONE-FLASK PROCEDURE
Storage
Donor
Exchange of cap
Recipient
Temporarily Open
Storage
Donor
Recipient
Completely Closed

unexpectedly costly and unsatisfactory. He must take into account the economics of mass production which makes items readily available at low cost. For example, if he should decide to have made a glass flask of new design, the expense of the mold will be charged to him at a cost of several hundred dollars. Rubber closures of special design are costly for the same reason. Every single piece of new equipment should be in commercial production for this or another purpose, or else the apparatus is likely to prove economically unpractical. This is not written to discourage experimentation with new equipment, because much development is badly needed, but the doctor with a new idea for blood transfusion apparatus should realize some of the obstacles to its development.

TRANSFUSION METHODS

It has been conventional to distinguish between *direct* and *indirect* methods of transfusion. This classification was developed in the early days of modern transfusion when there was considerable controversy as to the desirability of the addition of anticoagulants to blood. With the modern acceptance of the use of anticoagulants, such a distinction has lost its significance and will not be perpetuated here. The practice of storing and preserving blood for transfusion demands a more logical classification of transfusion procedures based upon the proximity or separation of the donor and recipient in space, and the duration of time between the collection of blood from the donor and its administration to the recipient. The following classification is therefore proposed, based upon the distinction between *immediate* and *delayed transfusion:*

Temporal Classification of Transfusion Methods

IMMEDIATE TRANSFUSION
- Vessel Anastomosis
- Cannula Anastomosis
- Vein-to-vein Connection by Tubing and Valve or Pump
- Paraffined Tube Method
- Multiple Syringe Method

DELAYED TRANSFUSION
- Two Flask Procedure
  - Collection of Blood in Open Container
  - Collection of Blood in Closed Container
- One Flask Procedure
  - Temporarily Open Systems
  - Completely Closed Systems

the donor and recipient were ligated after the operation. The procedure was based upon the assumption that citrated blood was contraindicated.

Multiple Syringe Method. Needles are inserted into the veins of donor and recipient. A 50 ml. syringe is connected to the donor's needle and filled with blood. It is then disconnected, fixed to the needle of the recipient, and the blood forced in. Sodium citrate solution may be placed in the syringe before starting and the instrument may be washed with sterile saline solution and used again. An assistant fills a second syringe while the contents of the first are being injected. This procedure is still suitable when small amounts of fresh blood are to be transfused, as, for example, in infants. The blood is necessarily injected at a faster rate than if an infusion flask were employed.

Delayed Transfusion Methods

In this type of procedure the withdrawal of blood from the donor can be separated from its injection into the recipient by hours, days, or weeks. The proximity of donor and recipient is not required. The addition of anticoagulant to the blood is necessary. The procedures can be employed for *fresh, stored,* or *preserved blood.*

Two Flask Procedure. Different flasks are used for the collection and the administration of blood. The chief purpose is to permit the filtration of blood through a filter surface which is larger than can be obtained in a flask or line filter. The blood is usually poured into an open filter just before it is injected. Any contaminating organisms are not given time to grow before the injection of blood into the recipient. The receptacle employed for the injection of the blood by gravity is frequently a calibrated Kelly bottle or salvarsan tube into which the blood is filtered. The top is closed with a loose metal cover or several layers of sterile cotton gauze. The advantages are: (*a*) there is rarely any difficulty with the mechanics of transfusion, even with large clots, because of the great filtering surface employed; (*b*) filtration is performed before injection is begun, so that extensively clotted blood is discarded; (*c*) stored or preserved blood can be filtered in the laboratory of the blood bank just before it is sent out to the hospital wards for transfusion. The disadvantages are: (*a*) the remote danger of infection of the recipient from contamination of the blood during filtration; (*b*) once blood is filtered it must be transfused within a few minutes or discarded; (*c*) the infusion receptacles are easily opened and are a constant invitation for careless persons to add solutions which hemolyze the donor's blood; (*d*) some infusion flasks will not contain a full unit of donor's

Immediate Transfusion Methods

In this procedure the withdrawal of blood from the donor and its entrance into the vein of the recipient occurs simultaneously, or within a few minutes. Anticoagulants may or may not be necessary. The procedures can be employed *only for fresh blood.* The donor must be near the recipient during the operation of transfusion. The various forms of apparatus used in this type of method will not be described in detail.*

Vessel Anastomosis. The artery of the donor was sutured directly to the vein of the recipient and the blood flowed by the greater force of the arterial pressure. The volume of transfused blood was estimated by the time during which the flow was permitted. This procedure was technically so difficult that it was seldom performed. The measurement of the volume of transfused blood was highly inaccurate.

Cannula Anastomosis. Various cannulas were devised by which the artery of the donor was connected with the vein of the recipient. This was a slight improvement on the technical facility of the operation, but otherwise it was similar to vessel anastomosis.

Vein-to-vein Connection by Tubing and Valve or Pump. A variety of machines have been invented by which the vein of the donor is connected to that of the recipient by tubing. The flow of blood is given direction in the tubing, either by use of a valve in the line which alternates the flow of blood to a receptacle from the donor and to the recipient from the receptacle, or by a pump or compressor in the line which forces the blood toward the recipient's vein. The apparatus is usually expensive, complicated to operate, and difficult to clean. Frequently the valves become stuck. Some valves have transmitted infection from the recipient to donor. The chief reason for the existence of this type of apparatus was the now out-moded prejudice against citrated blood.

Paraffined Tube Method. Cannulas were inserted into the veins of the donor and recipient who were in the same room. Blood was drawn by suction from the donor into a sterile paraffin-lined receptacle. The container was then detached from the donor's cannula, connected to the cannula in the recipient's vein, and the blood forced in under pressure. A period of approximately eight minutes was permitted for the collection and injection of a 250 ml. flask of blood before clotting began. The method was cumbersome and required the assistance of several persons. Usually the veins of

* [illegible] transfusion, the reader [illegible]nk and the Technique [illegible] 1942, Chap 15.

Completely Closed Systems. The blood may be collected by gravity with intermittent suction, or in an evacuated flask. In either case the container is stoppered with a thick rubber closure, the sterile surface of which is pierced with needles connected to the necessary lines of tubing. After collection the needles are withdrawn and the holes completely closed by the resilient rubber. During administration the closure is again pierced by a needle or adapter for the outflow line. The air vent is also made by puncture of the closure. The completely closed system is preferable for the transportation of blood and the use of expendable administration equipment.

ITEMS OF EQUIPMENT

This section comprises a discussion of the elements of the apparatus employed in the various methods of delayed transfusion which are adapted to the storage of blood: the two flask system with closed collection flask, and the one flask procedure, either with temporarily open system or completely closed technique.

CONTAINERS

Glass. For the storage of whole blood the National Institute of Health specifies the use of containers made from clear, colorless glass, Type I USP. The standard tests for the solubility of glass are described in the United States Pharmacopoeia. The solubility of glass varies, depending upon the use which it has undergone. Tests made under the direction of one of the authors showed that the amount of alkali dissolved from a given specimen becomes less with each successive heating with a solution. Therefore a container which gives off an excessive amount of alkali with the first autoclaving yields less with repetitions of the operation. This fact is of interest when refillable containers are employed for the manufacture of blood preservatives or infusion fluids.

Size and Shape. Commercially available containers for the collection and storage of blood range in capacity from 300 to 1200 ml., when filled to the top. The shape of those of 550 to 650 ml. has been well standardized to fit centrifuge cups having a diameter of about 9.8 cm. They are cylindrical with rounded shoulders and small mouths. The structure is designed to withstand a centrifugal force of 1700 times gravity, which is developed at 2500 revolutions per minute in the BP Model International centrifuge.

The larger flasks vary considerably in design. Containers from one source are cylindrical with rounded shoulders and total capacities of 600 and 1200 ml. Another manufacturer produces cylindrical flasks with shoulders like truncated cones and capacities of approximately 700 and 1200 ml. From another source flasks may

blood and a supplementary addition of blood must be made to the flask. This has resulted in confusion of blood flasks when two transfusions are being given to adjacent recipients.

Collection in Open Container. In this arrangement the blood is permitted to flow directly from the needle in the vein of the donor into an open receptacle containing sodium citrate solution and a glass stirring rod. Blood collected in this manner must be filtered and injected into the recipient immediately and the method is therefore employed *only for fresh blood.* If properly performed, the danger of infection of the recipient is not great. The method is unesthetic.

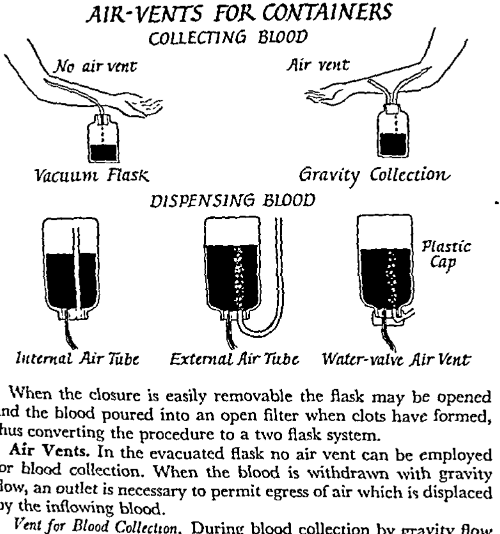
Collection of Blood in Closed Container. In this variation of the two flask method the donor's blood is collected in a flask by a system of tubing which does not permit access of unfiltered air to the container. The blood may then be stored in the collection flask for a time limited only by the preservative solution employed. Open filtration is performed just before injection of blood into the recipient. The method can therefore be employed for *fresh, stored,* or *preserved blood.* It is used particularly for blood in preservative mixtures in which there is much fibrin precipitation or clotting which would clog the filters in flask or line. The method is limited to hospital use because open filtration should be performed without subsequent transportation of blood any great distance.

One Flask Procedure. In this method the same flask is employed for the collection, storage, and administration of blood. During the injection of blood into the recipient the container is inverted. The procedure is esthetic, it is adapted for large-scale transportation, and there is a minimum of bulky auxiliaries, such as a large filter. A disadvantage is that the filters must be built into the flask and the line, seriously limiting their filtering surface. Therefore there are more mechanical difficulties with the one flask procedure than when two flasks are employed. The one flask method permits an entireiy closed system. Practically all apparatus manufactured with prepared preservative mixtures utilize the one flask procedure.

Temporarily Open Systems. The same flask is employed for the collection, storage, and administration of blood, but different closures may be used for all three purposes. The exchange of caps on the flask is usually performed immediately after the collection of blood and just before its administration. This constitutes one or two interruptions in the closed system but the manipulations can be made safely with aseptic technique. In this variation of the one flask procedure the blood is usually collected from the donor by gravity flow, with the option of employing intermittent suction on the air vent of the flask.

Before the collection of blood the cap is removed and the sterile temporary closure, such as a two holed rubber stopper, is aseptically placed in the mouth of the flask. After collection, this is replaced by a sterile screw cap or a dispensing cap which closes the flask during storage. The screw cap is then succeeded by a dispensing cap just before the injection of blood.

Another variation of the temporarily open system is the use of a bakelite cap with different detachable accessories which adapt it as a collection cap, a storage closure, and a dispensing cap. Plastics of the bakelite type are hard, insoluble in the solutions used in blood transfusion, and do not shrink or stretch with the heat of autoclaving.



When the closure is easily removable the flask may be opened and the blood poured into an open filter when clots have formed, thus converting the procedure to a two flask system.

Air Vents. In the evacuated flask no air vent can be employed for blood collection. When the blood is withdrawn with gravity flow, an outlet is necessary to permit egress of air which is displaced by the inflowing blood.

Vent for Blood Collection. During blood collection by gravity flow an air vent must be employed. With the permanent rubber closure a sterile assembly is employed consisting of a short piece of tubing attached to a needle. The other end contains loose cotton wool.

be obtained which are shaped like prescription bottles with oblong bases and capacities of about 700 and 1200 ml. Another firm supplies containers which are pear-shaped, with rated capacities of 500 and 1000 ml., although the total fluid space is somewhat larger.

The mechanical strength of the container depends more upon the shape of the flask and the thickness of the walls than upon the type of glass of which it is composed. This is principally of interest in the processing of plasma by centrifugation, and the flask specifications are therefore given in a discussion of that subject (p. 363).

Closures. The type of closure depends upon whether the air in the container is at atmospheric pressure or the flask is evacuated.

Closures for Vacuum Flasks. Vacuum flasks are invariably closed tightly with rubber stoppers in which wells and indicators are molded and through which needles and adapters are inserted for the collection and dispensing of blood. The rubber should be sufficiently pure so that particles are not suspended when the solutions are autoclaved or stored in the flasks. The resilience of the rubber should be such that repeated punctures of the stopper by a needle will not result in the deposition of plugs of rubber in the solution or in leaks in the closure after the needle has been withdrawn.

A simple test for vacuum closures can be performed if the evacuated flask is partly filled with fluid. A 16 or 18 gauge needle is securely fitted on a tightly closed syringe. The needle is repeatedly plunged through the closure at the point indicated for puncture. After each puncture and withdrawal, the flask is inverted to determine whether air bubbles rise from the point of puncture. A good closure should be able to withstand eight or ten punctures in the same vicinity without leaking and without the deposition of particles of rubber in the fluid. When the flask is inverted for dispensing, the proper orifice in the closure should fit tightly around the adapter so that the weight of tubing, filter, and other auxiliaries can be sustained.

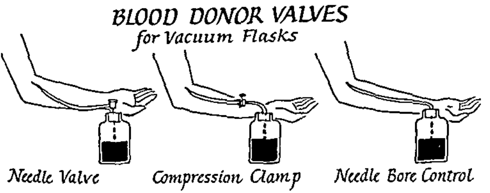
Closures for Nonevacuated Flasks. The design varies depending upon whether the system is completely closed or temporarily open.

In the completely closed system the closure may be punctured by a needle during blood collection and by another needle or adapter when the blood is dispensed. The stoppers, usually of rubber, are not necessarily so thick as those used in the vacuum flasks. The rubber should be resilient and nonfriable to withstand multiple punctures. When the container is inverted for dispensing, the closure must hold the inserted needle or adapter, weighted with tubing, filter, and accessories of the injection line.

In the temporarily open system the preservative solution is usually autoclaved and stored in a flask with a plastic screw cap.

their bases by which the inverted containers can be suspended during the administration of blood.

Vacuum in Containers. The vacuum flask for the collection of blood is usually prepared commercially and contains the sterile blood preservative solution for 500 ml. of blood. The vacuum should be sufficient to fill the flask almost completely with blood. If the vacuum is excessive, however, the tubing and the donor's vein will collapse during blood collection. The optimal vacuum is about 54 cm. of mercury. A sample container may easily be tested by determining how completely it is filled when water is permitted to enter through the blood collection apparatus. The evacuation of flasks on a small scale is not advised unless a good vacuum pump and a mercury manometer are available.



Blood Donor Valves

In using an evacuated flask for the collection of blood some device is necessary to control the pull exerted by the vacuum upon the vein of the donor. Three different principles have been used for this purpose.

Needle Valve. A needle on one arm of a metal needle valve is thrust through the rubber closure of the flask and the other is connected to the tubing from the donor's vein. The valve is then opened cautiously and the blood flows through into the flask. When properly made, these devices are expensive and difficult to clean. They are an integral part of the blood line and therefore must be sterile. This necessitates a separate valve (with several in reserve) for each donor bled during the day. Supplying these for a large donor service involves a considerable outlay of capital. Some designs have a number of small parts which are difficult to clean and intricate to replace.

Compression of Tubing. The flow of blood may be controlled by compression of the tubing from the donor's vein by several types of clamp, the simplest of which is the Hoffman screw clamp. This

The needle is plunged through the rubber closure aseptically. Intermittent suction may be applied to the flask by connecting the free end of the tubing with a tightly fitting syringe or rubber bulb with a one way valve. A similar assembly of tubing and air filter may be used with the bakelite collection cap. If a two holed rubber stopper is employed to collect blood, one adapter through the closure should be reserved for the air vent which can be attached to an air filter by tubing.

Vent for Blood Dispensing. Three types of air vents for dispensing blood from a closed inverted flask have been employed: (A) Internal Air Tube. An airway is established through the closure by means of a glass tube fixed in the rubber stopper and extending up through the surface of the blood mixture. An alternate method is to puncture the closure with a sterile needle which is long enough to reach above the fluid level in the flask. (B) External Air Tube. An inlet in the closure is made by a needle inserted in the rubber, or a channel is built in the plastic cap. This is connected to sterile tubing which is attached vertically outside the flask so that the open end is above the level of the fluid in the container. The air bubbles up through the column of blood mixture to replace the fluid which is injected. (C) Water-valve Air Vent. A short air channel is built into the plastic dispensing cap in such a manner that the ingress of air is readily accomplished but backflow of liquid is more difficult.

Each of these types have advantages and disadvantages. The internal air tube (Type A) and the external air tube (Type B) do not readily permit backflow when the flask is lowered during manipulation of the apparatus; this advantage is not possessed by Type C in which a little back pressure results in the ejection of blood through the air vent. The built-in glass tube in Type A sometimes becomes detached from the closure or is shattered during shipment; this failure does not occur with the other types. In some plastic dispensing caps, where the inlet and outlet are close together, the ingoing air bubbles tend to agitate the blood mixture near the outlet and prevent clots from plugging the orifice; this advantage is perhaps offset by the fact that the air may be channeled from the inlet to the outlet, instead of rising from the surface. Certain plastic dispensing caps tend to become air-locked more easily than in the arrangement in Types A and B. In Types B and C a flask of crystalloid solution may be hooked in tandem to precede or follow the blood intravenously, whereas a Y-tube connection is required in Type A (p. 537).

Bales for Containers. Most commercial flasks for the collection and dispensing of blood have attached metal or fabric bales on

Glass Cloth. Fabric made of glass fibers has been employed for expendable blood filters. It is more expensive than the other two mentioned but is somewhat more rigid. On theoretical grounds, one would expect glass fibers to be more harmful to the endothelium than cotton, if an analogy can be made with what is known in the case of quartz fibers.

Plasticized Fabric. This is a new development in which cloth is impregnated with a hard, insoluble plastic which withstands the heat of autoclaving. It has been employed to produce a filter which has sufficient rigidity to hold its shape when wet. It is expendable.[1]

Glass Beads. The solid glass beads employed in the chemical laboratory have been used as a coarse filter in the form of a loose mass. The interstices are large but they tend to entrap the larger clots. They cannot be used in commercial blood containers because they chip during shipping. They are rather expensive equipment, but they may be cleaned and reused many times.

Gas Mantles. The Welsbach type of gas mantle was employed as a blood filter by the British Army Blood Transfusion Service. The pores are coarse but the exact size is not available. They consist of sacs of cotton fabric impregnated with thoria and ceria. The extent to which these substances are carried into the circulation during transfusion and their fate in the body are unknown to the authors. They were used as expendable equipment.

Stainless Steel Cloth. This has been widely used in the United States. It is a rigid cloth made of stainless steel wire. It is insoluble and can be reused indefinitely. It is difficult, if not impossible, to clean mechanically and the protein must be digested with fuming nitric acid. Its ability to be reused offsets the increased initial expense.

Bakelite. This plastic has been used to some extent as a flask filter. There is no inherent objection to the material but the designs have been unsatisfactory.

Porous Rubber. Filters have been tried with this material but the designs have been unsatisfactory.

Size of the Pores of Blood Filters. Fresh blood will run through a fine mesh filter satisfactorily whereas stored or preserved blood, freed from large clots, will flow through the same mesh either slowly or not at all. The difference is due to the following: (*a*) small clots formed during blood collection tend to grow in size during storage; (*b*) precipitation of fibrin may occur in the supernatant plasma in the form of a veil or sheet; (*c*) the buffy layer, formed of leukocytes during sedimentation, tends to clog the fine meshes of filters; (*d*) after the erythrocytes have been stored they are not readily resuspended by casual agitation of the container but tend to adhere to one another.

is inexpensive and has the advantage of not requiring cleaning or sterilization since it is outside the sterile field.

Control by Diameter of Needles. A simple device, first suggested by S. O. Levinson, is the use of a short piece (30 to 45 cm.) of rubber tubing, to each end of which is attached a hose-hub needle of 17 gauge. One needle of the sterile assembly is inserted into the donor's vein. When the blood flows, the other needle is thrust into the flask through the closure so that the vacuum exerts its pull. When collection is finished, the needle in the flask is removed first. The gauge of the needles prevents undue suction on the system. Hardin found that collapse of the tubing could be prevented by employing a 16 gauge needle in the vein and one of 18 gauge in the flask.

Blood Filters

It may be stated without qualification that the greatest unsolved problem in apparatus for the one flask method of transfusion for stored and preserved blood is the development of a satisfactory blood filter. Slowing of the rate of injection, or complete clogging of the filter, is not uncommon with the best design available, so that transfusion is dangerously prolonged or is entirely thwarted. With the great resources and ingenuity of commercial firms, it seems certain that better filters will be devised when the medical profession demands them and clearly formulates logical specifications.

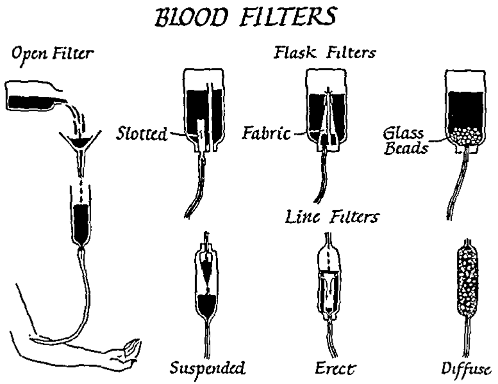
Composition of Blood Filters. Some of the materials of which filters are made at present are bleached cotton gauze, nylon fabric, glass cloth, plasticized fabric, glass beads, the Welsbach type gas mantle, stainless steel mesh, bakelite type plastics, and porous rubber. It seems likely that the filtration ability of the softer fabrics depends not only on their mesh but also upon the fine fibers which protrude from the threads and enmesh particles which reduce the size of the pores.

Bleached Cotton Gauze. The fibers swell when wet and tend to make the pores smaller. There is a prejudice against cotton because of the loose fibers which may be carried into the blood stream of the recipient. Little is known about the fate of such fibers in the body and less about the amount which enters during a transfusion. Suffice it to say that cotton has been employed to filter blood for many years and no proof has been offered of any harm from it. It is sufficiently inexpensive to be discarded after one transfusion.

Nylon Fabric. This does not shed as many fibers as cotton nor do the fibers swell as much when wet. It is more expensive than cotton. Filters of this material may be washed and reused up to eight or ten times.

the overall dimensions permissible are extremely limited. A recent design illustrates a method of increasing the surface without augmenting the overall dimensions. Formerly a stainless steel filter of cylindrical shape had been employed which was approximately 4.7 cm. in height and 1.6 cm. in diameter. Its filtering surface was about 27 sq. cm. Cooksey[1] devised a semirigid plasticized fabric of the same shape but 0.5 cm. longer. By deeply fluting the surface, however, the area was increased to about 48 sq. cm.

Sites of Filtration. In the administration of stored or preserved blood in the one flask completely closed procedure there are



actually two problems of filtration, one in the flask and the other in the outflow line. A finer filter is generally considered necessary in the tubing line although this is probably merely the result of convention. But before the blood mixture reaches the line filter it must pass through the outlet orifice of the container. Many blood clots will clog the line and completely obstruct the flow before the blood reaches the line filter. This obviously demands a mechanism in the flask which at least prevents the outflow orifice from being plugged. An efficient filter in the flask would obviate the necessity for a line filter but the reverse is not true. Nevertheless the medical profession has demanded line filters but not flask filters and it has been supplied accordingly.

The maximum size of the filter pores which permits free flow in the administration of blood is determined by the bore of the needle in the vein of the recipient. The minimal requirements of a filter certainly are that it should hold back particles which would plug the needle. However, the authors are aware of no scientific evidence to determine the optimum size of the pores of a blood filter. DeGowin has been repeated the following experiment many times. The coarse clots were first removed from a preserved blood mixture. The blood was then run through rubber tubing in which were three stainless steel mesh filters placed in tandem so that the uppermost was 60 mesh to the inch, the middle 100 mesh, and the lowest 200 mesh. After the blood had run through all three filters, they were examined. Particles of different sizes were caught in all three. Separately, a 200 mesh filter plugged often, a 100 mesh occasionally, and a 60 mesh infrequently. The total amount of debris held back by the three filters in tandem was usually less than one gram. The material was white or gray and quite friable, so that its fate in the circulation could only be surmised. It seems probable that the 200 mesh filter passed particles which were large enough to plug a pulmonary capillary.

On the basis of such experiments there seems to be no logical minimum size of pore which will withhold all debris and still function. The manufacturers have made filters of 100 mesh and 200 mesh because of criticism of coarser filters by the medical profession. It would seem practical to use the filters with larger pores unless scientific evidence is offered to contraindicate them.

Surface of Blood Filters. The efficiency of a filter depends as much upon the area of filtering surface as upon the size of the pores. If the amount of material to be held back exceeds the surface upon which it can be spread, regardless of the size of the pores, the filter becomes plugged and functionless and the recipient is prevented from getting the blood.

The amount of material held back in the filtration of 500 ml. of stored or preserved blood depends on (*a*) the size of the pores of the filter, (*b*) the preservative mixture, (*c*) the care exercised in blood collection, (*d*) the duration of storage of the blood. When the open blood filter is used there is practically never any difficulty about plugging it to the extent that it does not function. The one flask completely closed procedure, however, imposes a serious limitation on the filter surfaces and it is difficult to devise a design which will operate perfectly.

In the commercial manufacture of transfusion equipment the tendency has been to rely entirely upon a line filter, placed in a glass housing in the outflow tubing of the apparatus. At such a site

considered undesirable to transfusion. They are usually mounted in glass housings in the flow line from the blood flask to the recipient's vein. The housing is frequently designed to serve also as a flow indicator. If reusable, the ends of the housing should be plastic, rubber, or metal closures which can be removed for cleaning. Line filters may be suspended, erect, or diffuse.

(A) Suspended Filter. The filter is usually a sac or cylinder closed at one end, whose lumen communicates with the *inflow orifice* of the housing so that blood first enters the filter, passes through, enters the housing, and flows out into the line. Some filters are pliable, others rigid. Rigidity is accomplished either by a supporting structure or the material of the filter itself. Unsupported sacs tend to adhere to the sides of the housing when wet, so they cannot be employed as flow indicators. The rigid suspended filters can be designed so that the blood drips off and the drops can be counted. The advantages of Type A are that pliable fabric may be employed with minimum support, a flow indicator can be designed, and no air can get into the circulation when the filter is plugged.

(B) Erect Filter. This type of filter is made of rigid material in the form of a cone, cylinder, or semiovoid, whose lumen communicates with the *outlet orifice* of the housing. With this arrangement the blood flows first into the housing, then through the filter into its lumen, and finally through the outlet of the housing. Whereas one clot the size of the lumen may completely plug a suspended filter, it would merely remain in the housing if an erect type of filter were used. This type is not adaptable as a flow indicator so that a separate arrangement must be made for that purpose. *The filter must be completely immersed during the entire transfusion*, otherwise a partly plugged filter permits air to enter above the lowered fluid level on the filtering surface. Examples of this type are the gas mantle used by the British Army Blood Transfusion Service and the Cooksey filter of plasticized fabric.

(C) Diffuse Filter. Glass beads or particles of rubber may be placed loosely in a glass housing in the flow line. This usually produces coarse meshes and the upper layer of the material is rather easily covered and plugged.

Drop Indicators

If a drop indicator is not incorporated in the line filter a separate device for this purpose should be placed in the line of flow. The classic Murphy drop indicator has been widely used. It possesses the serious disadvantage of being extremely hard to clean. A design with removable ends is desirable so that there is easy access to the inside.

Open Filters. In the two flask procedure there are few difficulties with filtration. Perhaps this is the only excuse for this method of transfusion. The blood mixture is poured onto an open filter through which it runs into the infusion receptacle. The surface area of the filter can be made adequate no matter how much clot or fibrin veil is encountered. A common method is the use of a glass or metal funnel with a diameter of 15 or 20 cm. On it are placed three or four layers of bleached cotton gauze with a mesh of twenty or more threads to the inch. A supporting conical wire screen may be placed between the funnel and the cloth to keep the wet fabric from plugging the funnel. The entire assembly is, of course, sterilized before use.

Flask Filters. Most filters which have been constructed for this purpose have been made to withhold only the coarser material and depend on a finer line filter to hold back the remainder. There are three general types:

(A) Slotted tubes, perforated needles, or wire mesh tubes in the flasks have been attached to the outlet orifices in the completely closed system. These have been rather unsuccessful because of the small surface area.

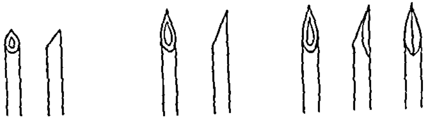
(B) A rigid structure supporting a cone of gauze in the flask was used by the War Emergency Medical Services of Great Britain. It was attached to the dispensing cap so that the blood passed through the gauze in running through the outlet orifice. The cap and filter assembly were inserted into the flask just before injection of the blood, making this a temporarily open system. In filters of this general design, in which the filtering tube protrudes for any distance into the blood mixture in the flask, a word of caution is in order. When the blood mixture has been lowered below the top of the filtering surface and the filter becomes partly plugged, *air may go through the filter in preference to blood and thus be carried into the vein.*

(C) Glass beads, loose in the container, make an excellent coarse filter. When the flask is inverted they settle in a layer over the outlet, providing many channels into the orifice. Some device is employed to prevent a bead from forming a ball valve over the orifice. Beads are obtainable in several sizes but the most suitable is 5 or 6 mm. in diameter. The number contained in a liquid measure of 30 ml. capacity is sufficient for the average sized flask. If beads are used in the completely closed system, they must be placed in the flask when the preservative mixture is manufactured on the premises. In the temporarily open system, the beads may be sterilized in a separate container and poured into the blood mixture just before injection. The beads are reusable.

Line Filters. These are intended to withhold the finer particles

gauges as a donor needle. The long bevel is usually put on the donor needles. In Great Britain the bayonet point is in vogue for donor needles. This type, in addition to a standard long bevel, has two additional bevels with intersecting planes on the under side of the shaft, making two cutting sides of the long bevel and a third cutting edge on the back. When properly sharpened, a large gauge needle with this point can be pushed through the skin with very little effort.

BEVELS OF NEEDLES



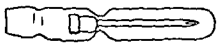
Short Bevel   Long Bevel   Bayonet Point

Sharpening of Needles. Dull needles of good quality may be resharpened or different bevels can be put on them. There are several devices available commercially but all require skill in operation. One of the simplest is a stone disc rotated by an electric motor. Only the rough grinding can be done by such a mechanism and the final polishing and honing must be performed by hand on an Arkansas stone or one of similar quality.

A simple test for the sharpness of a needle is to plunge the point through several layers of fine silk fabric. With a little practice the difference in resistance produced by a sharp and a dull needle can readily be distinguished. Hooked points may also be detected in the same manner.

Protection of Needles. Needles are easily dulled when packed without protection in transfusion equipment. A popular and efficient device for the storage of sterile needles is the constricted glass tube. This is a small test tube with a constriction in the wall near the open end. The shaft of the needle is inserted into the lumen but the point is prevented from touching the closed end by the constriction which

Constricted Tube



holds back the hub. The mouth of the tube is closed by a cotton plug or a rubber cap. Needles protected in this fashion may be packed in transfusion equipment or a supply may be kept separately.

NEEDLES

Much attention has been devoted throughout the history of transfusion to special needles for the veins of the donors and recipients. Many varieties are still made for these purposes. The relative merits of each are matters of opinion and frequently not susceptible to objective proof. The published diagrams showing the behavior of a certain type of needle as it enters the vein are largely hypothetical and not nearly as objective as they seem. It is noteworthy that most skilled transfusionists are indifferent to the specially designed needles but insist only that the needle be sharp and of proper size. The special designs appeal most to those who would try to substitute a new instrument for lack of skill. Only multipurpose needles of standard design will be discussed.

Quality of Needles. The metal and the workmanship are important considerations. Good needles are sufficiently expensive so that they should be reused. Only those of good quality can be resharpened satisfactorily. One of the tests of quality is to take a needle direct from the factory, deliberately dull it, and attempt to resharpen it. Good needles have a polished bore whereas poor ones may have a rough interior. The roughness promotes clotting during the collection of blood.

Hubs of Needles. Three types of hubs are available. The usual hub, of varied external design for grasping with the fingers, is reamed to fit the Luer taper and therefore can be affixed to glass observation tubes or Luer syringes. The hose hub is slightly bulbous to permit its insertion into rubber tubing. The inside of the hub is not reamed to fit a taper. The third type is a double purpose hub which is externally like the hose hub but is also reamed to fit a taper.

Gauge and Length of Needles. Standard needles are available in a variety of sizes but only a few are necessary in transfusion. For the collection of blood from adult donors a 15 or 16 gauge needle is usually preferred. The length most frequently suitable is either 1½ or 2 inches (3.8 or 5.1 cm.). Needles shorter than this are difficult to employ where there is much adipose tissue over the vein.

For the injection of blood 18, 19, or 20 gauge needles are preferable for adult recipients. The usual length is 1½ to 2 inches (3.8 to 5.1 cm.). Gauges from 20 to 24 are necessary in transfusions to children. The length is proportionately less. A 24 gauge, ½ to ⅝ inch (1.2 to 1.6 cm.) needle is frequently used in the veins of infants.

Bevels of Needles. Three useful types of bevels may be put on a needle during resharpening. They are the short bevel, the long bevel, and the bayonet point. The short bevel is preferred by many in the injection of blood but is not likely to gain favor on the larger

desired distance. A simple metal plate with two jaws formed by a cutout isosceles triangle is in use. The degree of compression of the tubing is regulated by the distance which the tubing is forced toward the apex of the triangle.

PLIABLE TUBING

The choice of pliable tubing for the administration of fluids parenterally has increased greatly in the last few years. Three general types will be considered.

Latex Rubber Tubing. The amber semitransparent type is preferred. Tubing is available which is manufactured especially for use in intravenous therapy so that it is free from acids and sulfur. The inside bore should be smooth and seamless. The standard sizes which are used for transfusion equipment are:

Bore in inches	1/8	1/8	3/16	3/16	1/4	1/4	5/16
Wall thickness in inches;	1/32	1/16	1/16	3/32	1/16	3/32	1/16

The thin walled tubing with 1/8 inch bore is suitable for expendable sets but it is too small for economical recleaning by hand. In our experience good grades of tubing will withstand fifteen or twenty autoclavings before they become soft, dark, sticky, and inelastic. The claim of some manufacturers that their products withstand seventy-five autoclavings should be carefully checked.

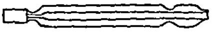
Vinyl Plastic Tubing. There are many variations of this type of plastic so that only a general description can be given. The material is white and translucent. It is pliable but somewhat harder than latex rubber. It can be molded by heat but the elasticity is much less than latex rubber. For this reason the tolerance of measurements between tubing and connections is small. Without special seals the tubing on these connections is liable to mold during autoclaving. Parting at the connection between tubing and metal or glass is not uncommon. In some types loops of tubing tend to adhere during autoclaving if special care is not taken in packing. The larger sizes are reusable and stand autoclaving as well or better than latex rubber. With repeated sterilization by heat the color of the plastic gradually turns to a red or brown, in some types. It also becomes brittle. It is used for expendable equipment with a bore of 1/8 inch and a wall thickness of 1/32 inch. The material can be made in a form which is nontoxic although most types contain considerable amounts of lead. The plastic is noninflammable.

Cellophane Type Plastic. Tubing of the cellophane type is available in sizes suitable for transfusion equipment. The walls are thin and transparent, but there is a marked tendency to kinking. A

Observation Tubes

These are short glass tubes with a slight constriction at one end for the attachment of rubber tubing and a ground Luer taper at the other, fitting the hub of a needle. The purpose is twofold: (*a*) it serves as a rigid handle for the needle when it is being inserted into the vein, and (*b*) it furnishes a window adjacent to the needle through which the operator can see the reflux of blood from the vein

Observation Tube

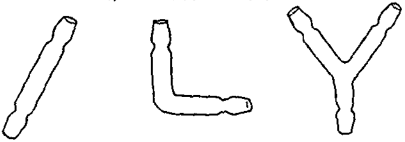


when the needle has entered the lumen. The smallest bore in the observation tube should be as large or larger than that of the needle to which it is attached. It is useless to employ a 15 gauge needle in a donor's vein if the caliber of the observation tube is 16 gauge.

Connection Tubes

Connection tubes are made of glass or metal. The ends of the glass tubes are slightly constricted for connections with rubber tubing and closures. The metal tubes may have ends similarly designed or they may be tapered to fit into other reamed parts.

CONNECTION TUBES



Straight *L - Shaped* *Y - Shaped*

Both ends may be of the same size, or one may be smaller than the other to permit joining two sizes of tubing. The tubes may be straight, L-shaped, T-shaped, or Y-shaped.

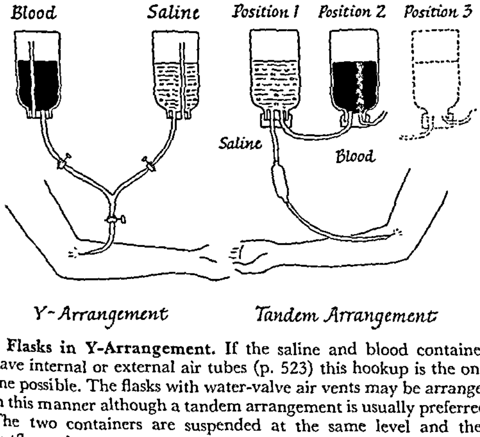
Tubing Clamps

In blood injection of the gravity type it is necessary to have a clamp on the tubing to control the rate of flow. A screw clamp of the Hoffman type is satisfactory. If a spring clamp is employed, it should have a screw adjustment which holds the jaws open at the

Combined Saline Infusion and Transfusion

Many transfusionists prefer to administer blood from a closed flask connected to another containing isotonic saline solution. There are three possible advantages: (*a*) the saline infusion may be started before the injection of blood to demonstrate that the system is functioning and the needle is in the vein of the recipient; (*b*) the infusion of saline solution may be started during a surgical operation and blood can be shunted into the line should the occasion require; (*c*) the patient may require an infusion of crystalloid solutions in addition to a blood transfusion. There are two methods of hooking up the blood flask and the saline container, depending on the accessories and fittings of the system.

COMBINED INFUSION & TRANSFUSION



Y-Arrangement *Tandem Arrangement*

Flasks in Y-Arrangement. If the saline and blood containers have internal or external air tubes (p. 523) this hookup is the only one possible. The flasks with water-valve air vents may be arranged in this manner although a tandem arrangement is usually preferred. The two containers are suspended at the same level and their outflow tubes are connected by a Y-tube from which one line leads to the vein of the recipient. There are clamps on all three limbs of the Y. The infusion may be begun with saline solution, shunted to the blood mixture, and back again, by manipulation of the various clamps. If a third flask of solution or blood is required, it is rather difficult to hook it onto the system aseptically.

single small puncture produces a permanent leak. The material is inelastic so that connections to glass or metal must be made with small tolerances of measurements. The tubing is connected to glass or rubber by winding with thread or the application of stretched rubber rings. For this reason the manual labor of assembling apparatus is much greater than when rubber tubing is employed. The plastic is unaffected by the heat of autoclaving and it is non-toxic. It is employed only as expendable equipment as it cannot be reused. Cellophane is porous and is employed for dialyzing membranes. When blood mixtures are run through it in transfusion, a small amount of water collects on the outer surface of the tubing from the dialysis of the blood plasma. This is of no consequence.

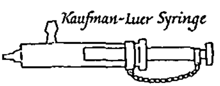
Attachments for Recipient Needles

There are several methods by which the needle in the recipient's vein may be connected to the line of tubing. Some of these are employed to observe the reflux of the recipient's blood when the needle punctures the vein.

Direct Attachment to the Line. The needle is attached directly to the tubing by means of a hose hub on the needle. Inserting the needle in the vein is a blind procedure with this arrangement. Attachment of the needle to a glass observation tube, which is fixed to the tubing, permits the operator to determine when the needle punctures the vein.

Syringe Exchanged for Tubing Set. If the tubing is equipped with an adapter or observation tube which attaches to the needle, the venipuncture may be performed with the needle fixed onto a syringe as a handle. When the vein has been entered, the syringe is disconnected and replaced by the adapter on the tubing.

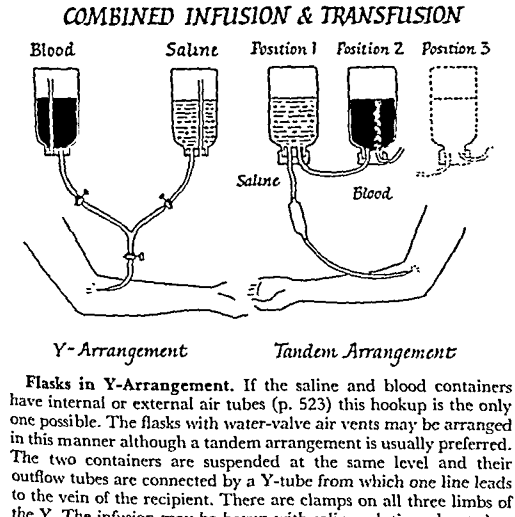
Kaufman-Luer Syringe. A special Luer syringe with a side arm may be employed. The side arm is attached to the tubing of the flow line and the recipient's needle is fixed on the taper in the usual manner. The plunger, closed before the vein is entered, is then



drawn back, bringing the recipient's blood, until the side arm is opened and the flow of blood is reversed. Clots occasionally form in the barrel of the syringe as the syringe is left in place during the injection of blood.

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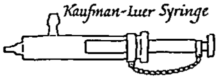
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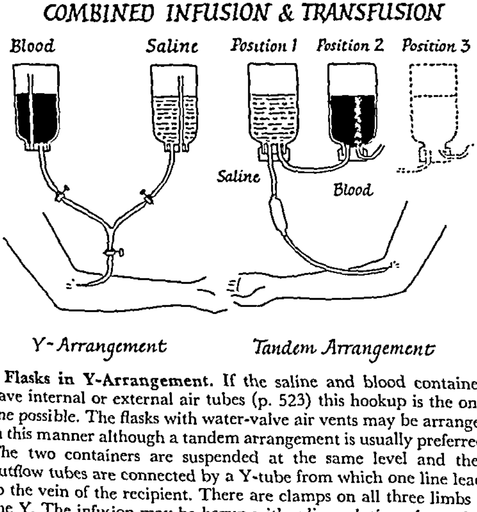
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Flasks in Tandem Arrangement. This system is only feasible with containers whose closures have water-valve air vents. Most of these vents are designed so that a slight rearrangement of the fittings will convert the airway into a fluid way for the attachment of another container. The bottles are usually hung at the same level, although occasionally it is advantageous to have the bottle farther from the needle at a higher level. In the tandem arrangement *the farthest flask empties first.* It is preferable to place the saline solution nearer the recipient (Number 1 position) and attach it directly to the tubing leading to the vein. The line filter for the blood is then in this assembly. When the injection of blood is desired, the leadout tube from the blood flask (Number 2 position) is attached to the modified air vent of the saline flask and the flow of blood is begun. The blood flows across the base of the saline flask, layering under the saline solution, and goes to the vein, in preference to the saline solution. Any number of flasks may be attached in the tandem arrangement.

DEVICES FOR ACCELERATING FLOW

If the system for administration of blood is not obstructed, the greatest resistance to flow is encountered in the bore of the needle. With a head of pressure of one meter (vertical distance between the needle in the vein and the level of the blood mixture in the flask) the flow of blood through an 18 gauge needle probably averages about 20 to 25 ml. per minute, against an average venous pressure in the recipient. The erythrocytes contribute much to the retardation of flow as shown by the observation that citrated blood flowed through an administration set and an 18 gauge needle at the rate of 26 ml. per minute, whereas blood in the dilute Rous-Turner mixture attained a velocity of 34 ml. per minute in the same apparatus with a similar head of pressure. The dilution of the erythrocyte suspension in the second solution accounts for the increased velocity.

The rate of flow of blood by gravity through an 18 gauge needle is sufficiently fast for most transfusions but it has been adequately demonstrated that in the treatment of severe hemorrhagic shock this velocity should be increased. The infusion can be speeded up by several mechanisms.

Increasing the Head of Pressure. This is the easiest maneuver to perform after transfusion of the recipient already has begun. However, it has relatively little effect in comparison with other methods.

Increasing the Bore of the Needle. Exchanging the usual recipient needle of 18 or 19 gauge for one of 16 gauge will result in a great

increase in the rate of flow. Velocities of as much as 49 ml. per minute with blood in the modified Rous-Turner mixture have been attained with a gravity system and a large needle.

Increasing the Pressure in the Flask. Many blood containers, especially those with the water-valve air vent, will not function properly if the pressure in the flask is increased. They spring leaks in unexpected places. For this reason it is preferable to apply the pressure in the outflow line between the line filter and the recipient needle.

Increasing the Pressure in the Line. This may be accomplished in either of two ways: (A) Syringe and Three Way Stopcock. A sterile assembly is inserted into the line. It consists of a three way stopcock attached to a 50 ml. syringe. On the hose connection is fixed a short piece of rubber tubing with an adapter which is reamed to fit a Luer taper. The observation tube of the transfusion set is removed from the recipient's needle and connected with the tubing on the stopcock. The Luer taper of the stopcock is fitted to the recipient's needle. The blood from the outflow line is alternately drawn into the syringe and forced into the recipient's needle under pressure. (B) Two Valve Syringe. A two valve syringe is inserted into the line in such a fashion that when the plunger is pulled out donor's blood fills the barrel, to be forced into the recipient's vein when the plunger is pushed. One satisfactory instrument for this purpose is the Hirsh-Adams bivalve syringe.

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1. Cooksey, W. B., and Puschelberg, G. C.. Disposable, fine mesh filter for blood and plasma. J.A M.A. 137:788, 1948.

CHAPTER 27

Preparation of Apparatus and Fluids for Parenteral Therapy

By ELMER L. DEGOWIN

Successful blood transfusion is dependent upon the use of equipment which is (*a*) chemically clean, (*b*) bacteriologically sterile, and (*c*) free from pyrogens. The chemical cleaning and sterilization of tubing, glassware, and other accessories are relatively well understood, but unfortunately the methods of freeing equipment from pyrogens and avoiding contamination with them are apparently unknown to many persons concerned with blood transfusion. Inability to carry out certain relatively simple procedures is leading to a policy in many institutions of using expendable infusion equipment and fluid containers. Economically this is a waste of material, much of which is actually reusable. The cost of this waste is eventually borne by the patient. It seems improbable that mass production of equipment can lower the cost to any further extent because most of the expense in modern apparatus for parenteral therapy is in hand labor for cleaning and assembling apparatus. With proper supervision and efficiency, labor can probably be obtained as cheaply by the hospital as in the commercial firm.

CENTRAL PREPARATION DEPARTMENT

The advantages of a central preparation department (frequently called a "central tray room" or "central supply room") for the cleansing, assembling, and sterilizing of all apparatus for parenteral therapy in the hospital have been well attested by many institutions. Special equipment can be installed for the use of full-time personnel, instead of entrusting the duties to untrained persons with inadequate

facilities on the ward units. In any special installation the preparation of apparatus for blood transfusion and intravenous infusion necessarily occupies much of the time and space.

ROOMS

For a large establishment in which both apparatus and fluids for intravenous therapy are prepared, *an ideal arrangement is a* series of contiguous rooms, each serving one of the following purposes: (*a*) receiving, (*b*) final cleansing and drying of equipment and bottles, (*c*) preparation of fluids, (*d*) sterilization of equipment and bottles, (*e*) distillation of water, (*f*) assembly of equipment, (*g*) dispensing, and (*h*) storage. In smaller institutions where all these functions are not performed, or the volume of work is not sufficiently great, many of the operations may be consolidated in different parts of one room. In any case the floor plan should be laid out only after a careful flow diagram has been constructed which shows all the procedures in the proper order. Operations which require steam lines should be grouped together as nearly as possible because of the cost of installation. Where heated air or steam is released into the room arrangements should be made to permit easy evacuation by exhaust fans. It is recommended that all doors be made wide to accommodate carts and trucks. The following discussion is based largely on years of personal experience in the State University of Iowa Hospitals.

Receiving Room. This should be located so as to be readily accessible from the corridors of the institution so that soiled apparatus and used bottles may be brought in by small wheeled vehicles. A large table is convenient upon which to disassemble apparatus. Large tubs, supplied by hot and cold water and compressed air, afford facilities for soaking and rough cleaning of equipment. Concrete floors containing drains will take care of spillage.

Washing Room. This should be supplied with softened hot and cold water, distilled water, compressed air, and steam. The floors should be of concrete, well drained. Along the wall range a battery of at least four contiguous tubs for washing. A table at one end of the line, over which are outlets for compressed air, serves for drying tubing. A steam operated bottle washer can be placed near the Fluid Preparation Room. Ample floor space is needed for parking carts of equipment and bottles. If steam is released into the room, an exhaust fan may be appropriately located. Smooth painted walls facilitate cleaning.

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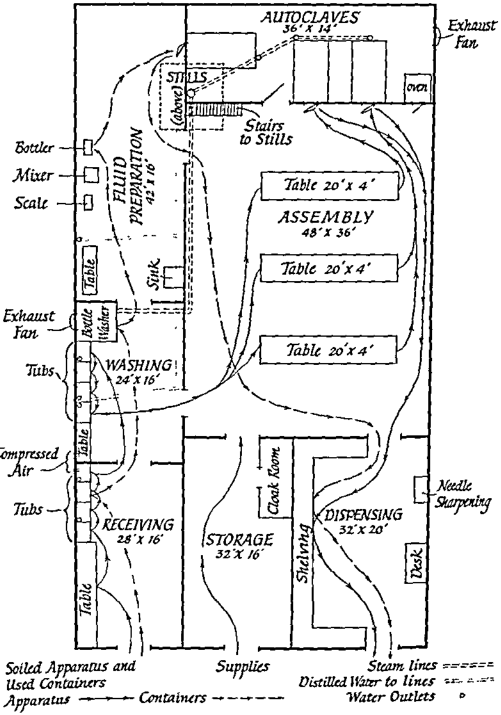
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Fluid Preparation Room. This should have a concrete floor with a good drain. It is necessary to have direct access to a large

autoclave suitable for sterilizing bottles of fluid. Floor space should be allowed for parking carts of bottles. Several long, wide work

SUGGESTED LAYOUT FOR CENTRAL PREPARATIONS DEPARTMENT



tables are desirable. On one a beam balance may be placed for weighing chemicals. A platform scale is necessary for measuring accurately large quantities of water for solutions. The filter, mixer,

and bottler are placed in a line along the wall. The walls of the room should be painted and smooth so that washing is easy.

Autoclave Housing. The autoclaves should be grouped in a housing whose walls are well insulated against heat. The autoclave for fluids should open into the Fluid Preparation Room while others for equipment should face the Assembly Room. It is advantageous to have the loading space in front of the autoclaves walled off from the Assembly Room to shield the latter from the heat radiating from the doors of the autoclaves. Ventilation of the autoclave housing is facilitated if it is located in a corner of the building with many windows opening to the outside. An exhaust fan can be installed in one of the windows. A large efficient fan, pulling the air to the outside, can also suck hot air through a grill in the wall of the housing over the autoclave doors, to take care of the steam which arises when the doors are opened. Floor space in the housing and a door into it must be made to permit working room for the steamfitters to repair and service equipment.

Distillation Room. A housing for the water stills is ideally built on the floor above the rest of the establishment. This serves two purposes. The heat of the stills will not gain access to the other rooms of the installation. More important, perhaps, is the fact that a gravity feed is possible for the distilled water lines to the rooms of the working area. It is economical to locate the stills over the autoclaves so that minimal lengths of steam lines are required. A staircase should lead from the working area directly to the still housing for convenience in control and inspection of these machines. An exhaust fan may be required to dissipate some of the heat given off in distillation.

Assembly Room. This should be commodious to accommodate large tables, many chairs or stools, and carts of materials and equipment. Smooth painted walls are desirable. Plenty of natural light, supplemented by overhead fluorescent lamps, is necessary. The floor should be covered with some resilient material, such as battleship linoleum, which is easy to clean and relatively soft to walk upon. Wall shelves should be avoided to facilitate the eradication of cockroaches and other vermin which might contaminate sterile equipment.

Dispensing Room. Logically this should have ready access to the corridors of the hospital and be close to the receiving room so that attendants may deliver soiled equipment and receive clean sterile packages in return. This is the natural place for the storage of sterile equipment and fluids. The walls should be lined with shelves upon which to store them. It is advisable that the shelves be relatively shallow so that they may be easily cleaned. This room

may be found convenient for a table where needles can be resharpened.

Storage Room. It is extremely convenient to have a storeroom in which supplies, such as bottles, rubber tubing, paper, linen, and other items may be placed awaiting demand. This can be a windowless room. It should open on the main corridor so that freight can be delivered without being taken through the other rooms of the establishment. One corner may be fitted as a cloakroom for personnel.

Equipment

In choosing equipment and fittings for a central preparation department several questions should be considered with every item: (1) Can it be easily manipulated by a muscularly weak person, such as a frail woman? (2) Can it be manipulated without much mechanical ability on the part of the operator? (3) Is it dangerous for one who has little knowledge of mechanics, chemistry, or physics? (4) Is it the most efficient method? (5) Are parts easily procurable and repairs readily made locally? (6) Is the cost excessive because the machine is custom-built or because it is made specifically for hospital use? (7) Can it be readily cleaned and dried so that pyrogens will not be formed? (8) Can the floor under it be readily cleaned?

In general, it is advisable to adapt machines which are in industrial use because they are more likely to be in mass production with the consequent advantages of being cheaper, more efficient, and having wornout parts readily replaceable. In the processing of fluids for parenteral use, this is particularly apropos. The manufacture of equipment specifically for the processing of parenteral fluids is an extremely limited industry. But, for example, the production of machinery for the dairy industry, which is concerned essentially with the sanitary handling of fluids, is highly developed and extensive. Whenever an easy adaptation can be made of dairy equipment, one is assured of a standard item, which is relatively cheap and for which parts for replacement are readily procured. Servicing is almost universally obtainable.

Washing Tubs. It is suggested that these be used in series of four for cleansing of rubber tubing and glassware. A convenient size is 24 inches wide, 36 inches long, and 18 inches deep (60 by 90 by 45 cm.). They are preferably made of stainless steel, with rounded corners, and seamless, or with smooth welded seams. They should be of 14 or 16 gauge metal. Each should have a drain which completely empties the tub. Special attention should be devoted to the attachment of the drain to the tub because this is

frequently the point at which leaks occur or bacteria accumulate in the crevices. They may be conveniently mounted on a frame of galvanized iron angles. Not more than two tubs should be mounted on a frame so that they are easily moved for repairs without bending the tubs. The adjoining top edges of the tubs may be covered with bent pieces of stainless steel to prevent dripping between them. They should be mounted so that an average-sized woman can reach the bottoms without flexing the spine. The bottoms should therefore be about 32 inches (80 cm.) from the floor. This is an important consideration in the efficiency of the personnel. It is convenient to use swivel faucets which can supply hot and cold water for two tubs and can be pushed out of the way. The faucets should be mounted on the wall and not on the tubs. A splash board should be installed back of the tubs on the wall.

Tubs suitable for the purpose may be in production for other uses or they can be manufactured to order by a firm making dairy equipment or kitchen accessories.

Dipping Baskets. These may be constructed of perforated sheet metal or wire. Copper or stainless steel is preferred. The latter is better but the cost is higher. A variety of sizes should be available for washing different items of glassware and plastics. It is desirable to have the baskets fitted with lids which sink to any level which they are packed. One or two long baskets may be employed for soaking rubber tubing. These should have handles because they are quite heavy when loaded.

Bottle Washers. No equipment can be recommended which will wash bottles of all shapes. If the bases of the containers are nearly round, brushes rotated by electric motors can be obtained which are designed for milk bottles. One arrangement consists of a single rotating brush for the inside of the container. Another utilizes a brush for the inside and another for the outside. These devices may be mounted on the edge of the wash tub. They require the separate handling of each flask and therefore are not efficient for large scale use.

When many bottles are to be washed daily, an attempt should be made to adapt standard dairy pressure bottle washers for the purpose. These accommodate a case of inverted bottles simultaneously. The pressure washers have three compartments containing respectively an alkaline wash, a rinse, and distilled water or steam.

Bottle Draining Racks. Special draining racks on small casters can be built to specifications by any carpenter. The wet clean bottles may be inverted to drain in the racks which can then be used as carts for storage and transportation around the establishment.

Tubing Cleaners. The cleansing of rubber tubing by hand is

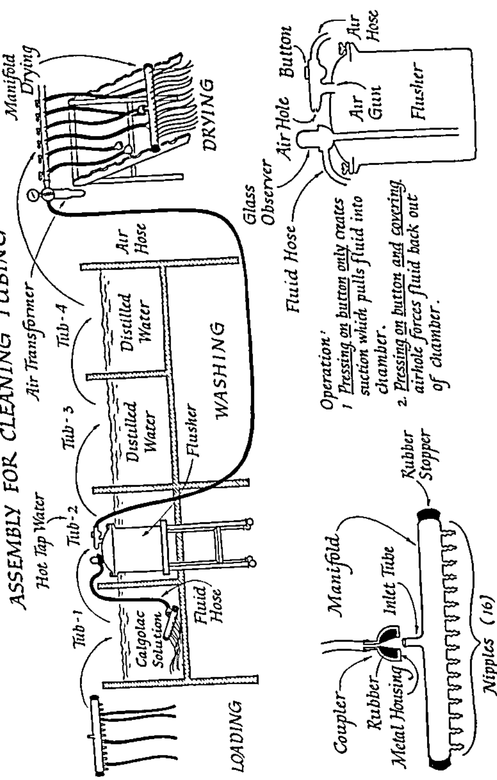
one of the most time-consuming operations in the central preparation department. For a small number of pieces, special nozzles may be fitted to water faucets and clots of blood forced out by streams of water or compressed air. Long pieces of pipe cleaner, procurable at laboratory supply houses, can be used for dislodging material which is especially adherent to the walls of the lumen.

For large scale cleaning, however, these methods are too slow. When 300 to 400 pieces of tubing are washed in a day, the manual labor is considerable. A machine which is in commercial production for the automotive industry has been adapted by DeGowin and has proved efficient and inexpensive. The essential elements are a series of wash tubs, several manifolds, an adapted flusher, and a compressed air line. The operation of washing with this device is described on page 558.

Flusher. This is an adaptation of an inexpensive item sold in the automotive industry as Model 78 Visi-Flusher (The Aro Equipment Corp., Bryan, Ohio) for the cleaning and flushing of automobile crank cases. It consists essentially of a steel sphere, holding about 6 quarts (5.6 liters), on top of which is mounted two accessories. One is an air gun, operated by compressed air, communicating with the sphere. The other is a standpipe, extending nearly to the bottom of the sphere and attached on the outside to a combined intake and outflow hose. When a button on the air gun is pressed, compressed air is forced through from pressure tubing and creates a vacuum in the sphere so that fluid is pulled in through the combined intake and outflow hose. With the compressed air still flowing, the air outlet on the gun may be closed with the thumb so that the force is directed into the sphere, reversing the flow of the fluid. Fluid can thus be forced back and forth in the system.

Several adaptations proved necessary. The steel sphere does not empty completely and the inside is inaccessible to cleaning. The steel rusts. The air gun and standpipe were mounted on the cover of an aluminum pressure canner (manufactured for domestic use) so that cleaning is easy and rusting is impossible. A canner with a capacity of about 9 quarts (8.4 liters) is preferable. The rusting standpipe was replaced by one of stainless steel. The entire apparatus was mounted on a small stand with casters so that the chamber is at approximately the same height as the tops of the tubs. The free end of the intake and outflow hose was fitted with a simple adapter which is manufactured for connecting a suction pump to a water faucet by the friction of a rubber gasket. As employed in this assembly, the adapter is used to connect the hose and the pipe of the manifold.

Manifolds. These were constructed in a local shop of pieces of



heavy gauge copper tubing about 20 inches (50 cm.) long and 1¼ inches (4.1 cm.) in diameter. At the center of the manifold a piece of copper tubing about 6 inches (15 cm.) long is attached by silver solder in the direction of a radius. The diameter of this tube must

fit snugly into the adapter in the inlet and outflow hose of the flusher. Along a radius, at a 90 degree angle from the connector tube, is soldered a line of brass hose connections (listed as *glands* in the catalogues of oxyacetylene welding equipment). The brass hose connections are obtainable in various sizes to fit the bore of the rubber tubing to be cleaned. The ends of the manifold are closed with laboratory rubber stoppers which may be removed so that cleaning is facilitated. It has proved most convenient to use at least eight manifolds, each with connections for sixteen pieces of tubing.

Compressed Air Installation. The main compressed air line carries a pressure of about 60 pounds (27.2 kilograms). At the outlet is mounted a standard fixture consisting of a filter which catches moisture and oil in the line, a reduction valve with two outlets, and a pressure gauge. One outlet is connected to the long line of pressure tubing leading to the air gun of the flusher. The other outlet is connected to a line containing six stopcocks with hose connections. The latter assembly is mounted over a table upon which is set a rack which holds a bank of six manifolds horizontally, one above the other. Short lengths of pressure hose with friction adapters connect the air line to the manifolds.

Fluid Mixing Tanks. Many containers are available which are especially designed for the mixing of pharmaceuticals and other fluids. Some are lined with glazed enamel which has a tendency to chip. Many are too large for mixing a lot of parenteral fluids. Although they are mounted on casters, some are easily tipped over when full.

Standard milk cans can be recommended as fluid mixing tanks. Those of good quality are seamless and lined by repeated dippings in tin. They have the added advantages of being easily handled, they can be sterilized by heat, and are easily procurable at moderate cost. A 10 gallon size (37 liters) holds 35 liters of fluid for careful mixing. Five gallon sizes (18.5 liters) are available for smaller lots.

Scales. For the preparation of fluids two sizes of scales are necessary. A table balance, with two calibrated beams and a tare beam, which weighs up to 5 kilograms to an accuracy of 1.0 gram, is preferable for weighing chemicals employed in the manufacture of fluids.

There is no easy or accurate method of measuring a lot of 35 liters of water by volume and therefore it should be weighed. A large platform scale is necessary for the purpose A bench dial scale manufactured for weighing cheese has been found very satisfactory. The dial registers up to 250 pounds (113.6 kilograms) in increments of ¼ pound (about 112 gm.). The built-in beam balance is used

as a tare scale for the weight of the container. The can is set on the platform, the tare is counterbalanced, and distilled water is run in until the proper weight is registered.

Fluid Mixer. A means is necessary to mix thoroughly and dissolve the powdered chemicals which are added to the water in the preparation of the solutions. This is best accomplished by an electric motor mixer. The dairy industry and the pharmaceutical manufacturers employ a type which may be mounted permanently on a tank or a portable design which may be clamped to a tank or other structure. A shaft on which are mounted two propellers is preferred. For mixing solutions in milk cans the machine has been mounted on a vertical platform which slides up and down a track with counterweights. The milk can is rolled under it, the propeller shaft is immersed, and the solution is mixed. It should be specified that the motor have bearings for a vertical mounting if it is to be used in this way. The shaft and propeller blades should be made of a metal which will not corrode in sodium chloride or dextrose solution.

Fluid Filter. The filtration of large volumes of fluid by gravity is impractical, so either suction or pressure must be applied to accelerate the procedure. Since the problem is common in industry standard equipment is available for the purpose. A bench model filter driven by a pressure pump has been found suitable. Four sheets of asbestos filter paper are used of the grade employed in the filtration of whiskey, wine, and cider. It filters at the rate of about 3 gallons (11 liters) per minute. Filter sheets are available which are stated to be "pyrogen-retentive" but these have not been necessary. Models of filters may be obtained which can be sterilized by steam-generating units or by autoclaving. Sterilization of the filter has not proved necessary when the filter is thoroughly cleaned and dried after using. It should be washed with pyrogen-free water before filtration is performed. An attachment containing a stone filter is usually necessary to prevent the asbestos fibers of the filter pads from appearing in the filtrate.

Bottling Machine. If many bottles are to be filled with solutions, much time and labor is saved by the use of a bottling machine. Two types are available for the quantities likely to be handled in a central preparation department. The gravity feed type is employed in small dairies in bottling milk. The design is not readily adaptable for filling bottles with solutions because there is no method by which the container can be filled only to a predetermined level, other than the top.

Portable vacuum bottle filters are manufactured for industrial purposes. A suction pump creates a vacuum in the container when

the nozzle is firmly fixed on the lip of the bottle and the fluid is pulled into the container to a predetermined level, the excess being discarded. Two or three bottles may be filled simultaneously. One type operates by a hand nozzle when the bottles are placed on a table, another is mounted in a cabinet and the valves are tripped by the foot. In purchasing such equipment attention should be given to the ease of cleaning and the availability of parts for replacement.

Labels for Fluid Bottles. The number of labels required for solution containers in a hospital is not sufficient to justify automatic labeling machinery. Gummed labels have been found satisfactory. They may be printed on long rolls which fit a standard machine used for dispensing gummed tape in retail shops. Pressure on a forward lever advances the strip and wets the gum, release of the lever cuts off the strip. With a little practice the operator can cut off labels quickly at the proper place. Besides the necessary text on the label, colored inks have been employed for various isotonic solutions and black ink for those which are not isotonic with blood. A different shape of bottle is advisable for distilled water so there is less chance of injecting it intravenously through error.

The gummed labels must, of course, be affixed to the flasks after autoclaving. In making small lots of several solutions which are sterilized together, metal tags attached to the bottle necks by chains should be employed until after heating. No difficulty has been encountered in mislabeling fluids when a lot of one solution is sufficiently large so that two kinds of solutions are not processed at the same time.

Autoclaves. The horizontal type of autoclave is preferred for a central preparation room. It is most conveniently operated by steam. An arrangement is desirable by which the air can be evacuated by steam suction before sterilization begins and the wrappings and equipment can be dried afterwards.

A special design is available for the sterilization of a large number of bottles of fluids. It is a hexahedron with square ends and contains a double-decked metal rack which rolls in and out on a track and a special cart. It may also serve for other types of equipment, whereas the usual cylindrical autoclave is poorly adapted for bottles. All autoclaves should be equipped with continuous temperature recording devices.

Dry Heat Oven. An oven operated by gas or electricity will be found invaluable in the drying of glassware and the sterilization of glass and metal equipment. Metal cutting edges are better preserved by dry heat sterilization. A domestic cooking range is inexpensive and efficient, and servicing is readily obtainable.

Water Stills. Triple distilled water is unnecessary for the manufacture of parenteral fluids. Water from a single still of good design is free from pyrogens and other noxious substances, if the machine is properly operated. It is preferable that water for distillation be soft, as hard water fouls the stills much more quickly. Different designs are required for heating with gas, steam, and electricity. There is a much greater range of sizes available in steam-operated stills than in the other types. Satisfactory steam stills may be obtained with hourly capacities of from 5 to 50 gallons (18.9 to 189 liters). Gas and electric stills are usually made in only the smaller range of sizes.

Some steam-operated stills are designed with a shunt by which the steam which heats the coils is then condensed to water which passes through the still as distillate. This obviates the necessity for a supply of soft water and actually constitutes a double distillation, since the water was first distilled when made into steam. A supply of cold water is necessary for cooling, but this may be hard. The operation of the still may be made completely automatic by means of suitable electrical relays and switches.

Storage tanks for distilled water are available in horizontal and vertical cylinders, and in many sizes, depending on the needs. One is necessary and a second is convenient. The tanks may be fitted with steam coils for heating, which inhibits the growth of bacteria and molds.

Pipes for distilled water lines are available in three materials. The conventional material was formerly block tin, but this is very expensive. Pyrex glass is also available with special flanges and joints. This is nonreactive and satisfactory, but also costly. Aluminum pipe is entirely suitable for water to be employed for intravenous fluids. It is less expensive than the other two types. The water forms an insoluble aluminum oxide which protects the metal and the fluid. Aluminum pipe is easily installed.

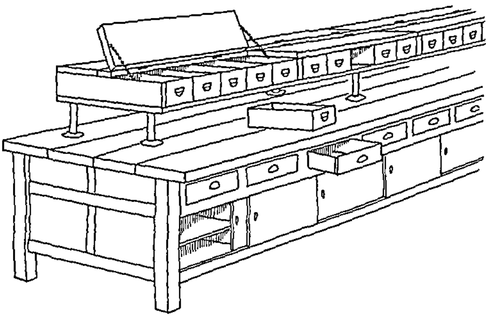
In planning the installation of stills several facts should be considered. Steam pipes are expensive and they should therefore be as short as possible. The distilled water lines should have gravity feed and be as short as convenient because they must be periodically cleaned. Bleeder valves at strategic points in the line will aid the emptying during cleaning. All pipe lines for pyrogen-free water should be detachable in 8 or 10 foot lengths for frequent cleaning.

Assembly Tables. Most of the work of assembling equipment must be performed by hand so that the only means of improving efficiency and diminishing labor lies in providing motion-saving devices.

An assembly table has been in service for several years which

has been convenient and time-saving: The table is 4 feet wide and 20 feet long (1.2 by 6 meters). It is supplied with a tier of drawers beneath the surface, and below that is a row of cupboards with sliding doors. The design is duplicated on the other side. Elevated above the surface of the table by iron posts is a superstructure, the edges of which do not project to the edges of the table. The superstructure holds a bank of small removable drawers, grouped in fives by partitions. Each section is covered by a hinged cover which may be held open by a stop of chain or leather. When resting on the drawers the cover makes a temporary working surface. The underside of the covers are studded with wooden pegs so arranged that when a drawer is pulled open, with the cover down, a peg serves as a stop to prevent complete withdrawal. This is for the purpose of opening a single drawer to insert or withdraw one or two objects.

ASSEMBLY TABLE



The drawers may be removed singly to be filled. Each holds one type of item. All drawers containing items for a single assembly are grouped together on the superstructure. When sets are to be assembled, the trays are placed on the main table under the drawers of the superstructure containing the proper items. The covers to the drawers to be used are then opened. Heavier equipment is placed in the drawers and cupboards beneath the table.

Table Coverings. Surfaces of tables which are frequently wetted should be covered with stainless steel or monel metal. Several

different materials have been tried for tables upon which hot bottles are laid. Soft wood is unsatisfactory because of splintering. Pressed wood has given good service but is badly scratched by the edges of bottles. Stainless steel is preferable. Battleship linoleum is suitable to cover the assembly tables. Any table covering should be sealed tightly to the underlying surface so as to provide no refuge for insects.

Carts and Movable Platforms. Carts with rubber-tired wheels can be adapted for the transportation and temporary floor storage of supplies and materials used in the department. A supply of small wooden platforms, each with four rubber-tired casters, will be found convenient for the moving of filled milk cans and the storage of drums of chemicals. Placing all possible equipment upon wheels facilitates the cleaning of floors.

PYROGEN-FREE DISTILLED WATER

The entire practice of modern parenteral therapy is built upon the knowledge of the characteristics of pyrogens and the methods of preventing their injection into patients. The preparation of apparatus and fluids for intravenous use is absolutely dependent upon adequate supplies of distilled water which is pyrogen-free.

Characteristics of Pyrogens

Source. Many nonpathogenic bacteria found in river water and on dust grow sufficiently in water to produce soluble carbohydrates which cause chills and fever when injected intravenously or subcutaneously into man or animals (p. 266). If the organisms are introduced into distilled water, which is initially pyrogen-free, the bacteria must grow from fifteen to eighteen hours before sufficient pyrogens are formed to cause febrile reactions. Bacteria need moisture for multiplication and therefore articles which are perfectly dry are not likely to support bacterial growth.

Action of Heat. The pyrogen-producing bacteria are killed by exposure to steam at 121° C. (250° F.) for twenty minutes or dry heat at 180° to 200° C. for sixty minutes. However, this amount of heat *does not destroy pyrogens* which have been previously produced. It is therefore not sufficient to sterilize apparatus and fluids; the sterilization must be performed *before the organisms have had time to grow and produce their products.*

A nonbacterial source of pyrogens is the plasma proteins in apparatus for parenteral therapy left from imperfect cleaning. The protein is denatured by the heat of sterilization. Some molds and bacteria become adapted so that they grow and form pyrogens in storage tanks where the water is kept at 70° C. or more.

Solubility. Most pyrogens are readily soluble and therefore may be washed from apparatus with sufficient pyrogen-free water.

Distillation. Pyrogens can be removed from water by distillation or by adsorption on certain types of filters. An efficient water still is most satisfactory for the purposes under consideration.

Testing for Pyrogens. The only recommended method is the biologic test (p. 390). At least five healthy rabbits are used, each weighing at least one kilogram. The rectal temperature should be measured on four occasions at two hour intervals, from one to three days before the tests. Animals should not be employed whose rectal temperature registers 39.8° C. or above. No food is permitted during the periods when temperatures are being recorded, but free access to water is allowed. The animals should be kept in separate cages and should not be excited during the period of temperature observation.

The fluid to be tested is warmed to approximately 37° C. and 10 ml. is injected into the ear vein of each rabbit fifteen minutes after a control temperature reading is taken. The temperature is measured for three successive hours after injection. The test is considered positive if three or more rabbits show rises of 0.6° C. or more. Some lots of pyrogens will produce chills and fever in human beings when the biologic test is negative.

Testing the conductivity of the fluids or the performance of chemical tests will not indicate the presence of pyrogens.

Manufacture of Distilled Water

Water for parenteral solutions and for washing pyrogens from equipment is usually prepared by distillation. A single type still is adequate if protected by spray-trapping devices, multiple baffle plates, and deconcentrator tubes so that the water does not leak or boil over into the distilled water lines (Rademaker, L.: Ann. Surg. *92*: 195, 1930). Once pyrogens have been removed by distillation, care must be taken to insure against bacterial contamination along the line. The following precautions are necessary: (A) *Use Freshly Distilled Water.* Water should not be employed for the making of solutions or the rinsing of apparatus which has been distilled for more than eight hours, unless it has been stored in hot tanks. (B) *Avoid Reflux in the System.* The storage tanks and lines from the stills should be so designed that at no point is there a possibility of flow of water from a contaminated region to one which is supposedly clean. (C) *Clean Tanks and Lines Regularly.* Molds and bacteria may form colonies on the walls of storage tanks and pipes. Most storage tanks are constructed with wide openings through which they may be cleaned manually. The pipes must be cleaned

chemically or by steam. It is recommended that the storage tanks and lines be filled once weekly with a solution of one of the hypochlorites in a dilution which will yield at least 100 parts of chlorine per million of water. This is permitted to stand for one hour and then drained off. The system is then flushed with freshly distilled water until chlorine can no longer be smelled or tasted in the water. In our experience this method cannot be relied upon. The tanks and short lengths of pipe should be disconnected and thoroughly scrubbed weekly to remove molds and other fungi.

PREPARATION OF APPARATUS FOR PARENTERAL THERAPY

The preparation of apparatus for blood transfusion, plasma infusion, the infusion of crystalloid solutions, and hypodermoclysis may be divided into several operations: (*a*) cleansing, (*b*) assembling and packaging, and (*c*) sterilization.

Cleansing

This operation consists of (*a*) washing mechanically and chemically, (*b*) rinsing with pyrogen-free water, and (*c*) drying.

Preliminary Washing. Infusion apparatus which has been in contact with blood can be more easily cleaned if the blood is washed out with *cold* or *lukewarm* water immediately after the equipment has been used. This can best be accomplished by the nurses in the various units of the hospital where the apparatus has been soiled.

Disassembling. When the soiled injection apparatus is returned to the central preparation department all elements must be separated from one another. Adapters, observation tubes, clamps, blood filters, and drip indicators should be disconnected from rubber tubing. Blood filters, syringes, and drip indicators are taken apart; needles must be removed from adapters and tubing; containers are opened and flask filters removed. As each item is removed from the set it should be placed in a separate dipping basket reserved for those of similar kind. The pieces which have been in contact with blood should be separated from those which have not.

Soaking. Pieces of equipment which have been soiled with blood should be soaked for an hour or more in water, or preferably 0.9 per cent sodium chloride solution, at room temperature. *Care should be taken that water completely fills the interior of hollow tubes of rubber and glass.* All loose blood clots can be washed out from tubing and glassware with a stream of cold water. Hollow glass and plastic articles can be stacked vertically in appropriate dipping baskets so that an up-and-down motion of the basket will force fluid through them. Latex rubber tubing should be filled from one end

to the other with fluid and then weighted, otherwise it will float from the buoyancy of the rubber itself and the contents will be emptied. Needles are soaked and then cleaned by forcing water and then ether through them with a syringe. Stylets should be employed to open plugged lumens. Special devices are available to release plungers which are stuck to the barrels of syringes.

Washing. All equipment, with the exception of needles, is washed in four solutions, preferably in adjoining tubs. Tub 1 contains detergent solution; tub 2, hot tap water to which a few drops of concentrated hydrochloric acid has been added; tubs 3 and 4 should contain pyrogen-free distilled water.

Many formulas are available for the detergent solution and detailed information on washing solutions is available from firms which manufacture dairy equipment, as extensive studies of the problems of washing have been made in that industry. Two formulas have been used by the authors with success:

Phosphate Mixture (courtesy of Mr. Charles T. Smith, State Hygienic Laboratory, State University of Iowa)

Powdered trisodium phosphate	amount in 100 ml container
Powdered tetrasodium pyrophosphate	amount in 30 ml. container
Hot water	1 gallon (3.7 liters)

Calgolac (Calgon, Inc., Pittsburgh, Pa.)

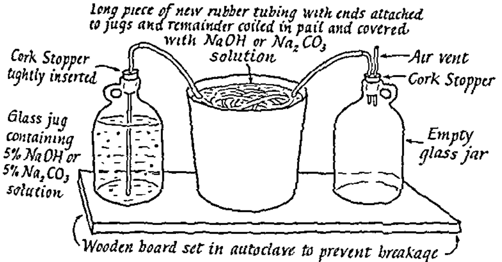
Powdered Calgolac	amount in 100 ml. container
Hot water	1 gallon (3.7 liters)

Washing Small Glassware and Bakelite Plastics. Small articles are packed in dipping baskets. Hollow tubes should be placed vertically so that they are completely filled on immersion. The original packing for soaking can be retained and the baskets merely transferred to the series of solutions for washing. The baskets are dipped and raised several times in each of the various solutions, beginning with Tub 1 and following the sequence. The glassware and bakelite articles, still in dipping baskets, may then be dried in a dry heat oven. Chemically clean glassware is not only free from visible foreign material but, when wetted with distilled water, the film recedes from a vertical glass surface in a more or less continuous line, whereas the line is broken and irregular if grease is present.

Processing New Rubber Tubing. Some manufacturers produce rubber tubing for parenteral use which requires no preliminary preparation. Other products come from the factory having sulfur and other impurities upon the surface which must be eliminated before use. This is best cleaned by subjecting the tubing to the action of hot 5 per cent sodium hydroxide or sodium carbonate solution. The procedure can best be performed on long pieces of tubing

before it has been cut into the lengths used for infusion apparatus. Some tubing comes in 50 foot lengths (15 meters) and the entire piece can be cleaned in one operation. A simple and effective method utilizes two one-gallon (3.7 liter) glass jugs and a large enameled metal pail. One jug is nearly filled with cleaning solution and closed with a tightly fitted one holed cork stopper through which a glass standpipe reaches nearly to the bottom. One end of the rubber tubing to be processed is connected to the end of the standpipe which protrudes from the cork. The other end of the

ASSEMBLY FOR PROCESSING NEW RUBBER TUBING



The assembly is autoclaved at 15 pounds (6.8 kilograms) pressure for 10 to 15 minutes, the pressure is then slowly reduced to atmospheric during 6 minutes, the door is opened from 5 to 10 minutes thereafter. The assembly is not removed from autoclave until the pumping of solution from the full to the empty jug has ceased.

tubing is connected to one of two short glass tubes piercing the cork in the other glass jug which is empty. All of the tubing, except the ends, is coiled in the pail set between the two jugs. Cleaning solution (sodium hydroxide or carbonate) is poured into the pail to cover the coils of tubing. The entire assembly is placed in the autoclave on a wooden board and sterilized at about 121° C. (250° F.) for ten to fifteen minutes. The pressure is then slowly permitted to drop to that of the atmosphere through a period of not less than six minutes and the door is not opened for another five to ten minutes. The assembly is not removed from the autoclave until the pumping of cleaning solution from one jug to the other has stopped. The tubing may then be washed with tap water and cut into pieces of suitable length and cleaned in the method described for used tubing.

Washing of Used Rubber Tubing. If the washing equipment described on page 546 is employed, the procedure is as follows: One person loads and unloads the manifolds with pieces of rubber tubing, another operates the flusher. A loaded manifold is attached to the hose of the flusher and immersed in Tub 1 so that the manifold lies at one end of the tub across the width and the tubing lies along the length of the tub. The tubing is weighted with a metal screen or pan so that it remains immersed. The fluid is drawn into the chamber of the flusher through the rubber tubing until the chamber is full. The flow is then reversed and the fluid is expelled back into the tub. The loaded manifold, still attached to the flusher hose, is then transferred to the next tub and the operation repeated there and in Tubs 3 and 4. The loaded manifold is then detached from the flusher hose, placed on the rack with the tubing dependent, and attached to a hose from the compressed air line. Air is permitted to flow through the loaded manifold for drying while other manifold loads are being processed. After drying the tubing is carefully transilluminated in a strong light to detect dirt or water in the lumens. The tubing may then be stored in a dry dustless container until needed. The chamber of the flusher should be opened, rinsed, and dried every day after using. The manifolds and hose should be cleaned and dried after the washing process is finished.

The question was raised as to whether there is a significant carry-over of pyrogens from Tubs 1 and 2 through the drying process. Measurements bearing on this were made. Each tub was filled with about 15 gallons (55.5 liters) of water. The carry-over of water on several manifolds, which were emptied and drained but slightly, was less than 50 ml. each, by weight. Therefore the carry-over from *Tub 1* was diluted in *Tub 2* 1110 times, in *Tub 3* 1,232,100 times, and in *Tub 4* 1,367,631,000 times. If, for some reason, *Tub 2* (containing tap water) became contaminated with 1 milligram of pyrogen (a high concentration), the carry-over to the drying stage would be 8×10^{-4} microgram. Measurements of the amount of sodium chloride left in the loaded manifold after draining, compared to that after drying, showed that the amount deposited on the dried tubing was one-fourth or one-fifth that in the wet tubing. If pyrogens follow the same reduction in drying, the hypothetical 8×10^{-4} microgram would further be reduced to 2×10^{-4} microgram. Co Tui *et al.* (p. 267) have estimated that the minimum pyrogenic dose of typhoid pyrogen is 0.02 microgram per kilogram for man, or 0.14 microgram for a 70 kilogram person.

This method of washing and dilution has proved adequate to clean rubber tubing so that pyrogenic reactions have not been observed clinically when the procedure was properly performed.

Rarely has clotted blood or coagulated protein been found in the tubing after washing.

For a small number of pieces of tubing the mechanical devices are unnecessary in washing. The fluid may be forced through the tubing with large glass or metal irrigating syringes, or the fluid may be aspirated through the tubing by a suction pump attached to a water faucet.

In any case, the tubing should be dried with filtered compressed air immediately after washing with pyrogen-free water, or else it should be autoclaved wet within six hours after washing with pyrogen-free isotonic saline solution.

Assembling and Packaging

After cleaning and drying, all articles of equipment are distributed to their proper receptacles on the assembly tables. When a certain type of set is to be assembled, metal trays are spread out on the large table and the sets are assembled and packed in the trays.

Metal Trays. Trays of enameled steel, aluminum, or stainless steel are convenient for containing a set of equipment employed in parenteral therapy. The trays are shallow but the edges should project slightly above the contents. It is desirable to employ as few different sizes as possible. The oblong shape is preferable because it permits easy wrapping and occupies a minimum of space on shelves.

Wrapping. After much experience, kraft paper has been found to possess many advantages over cotton cloth as wrapping material. It forms a covering which is more impervious to dust. The procedure of wrapping can be performed with greater speed. The paper dries well in an autoclave with a drying attachment. The label denoting the contents and the date of sterilization can be placed on the paper with a rubber stamp before autoclaving and the ink is not effaced by the steam. The edges of the wrapper can easily be fastened by wire staples affixed with a hand stapler. The paper wrappers are, of course, expendable.

A large quantity of paper for wrapping is previously cut to the desired sizes. The oblong tray is placed in the center of a sheet and the long sides of the paper brought together and folded over twice to form a double seam which is nearly dust-proof. The ends are folded at angles and stapled in place. No gaps in the wrapping should be left to allow the entrance of cockroaches which would contaminate the set.

Sterilization

The wrapped trays of apparatus are stacked in the autoclave in a crisscross fashion to permit maximum access of steam. The auto-

clave is run at 121° C. (250° F.) for twenty minutes. The drier is then turned on until all the steam has been removed from the paper.

PREPARATION OF PARENTERAL SOLUTIONS

The successful manufacture of solutions for parenteral injection depends upon (*a*) clean pyrogen-free containers and closures, (*b*) pyrogen-free distilled water, (*c*) pyrogen-free chemicals of C. P. grade, or better. The discussion will concern the preparation of quantities of fluids which are used in a large hospital. The same principles apply to the production of smaller volumes, but less machinery and smaller devices are required in the latter case.

Weighing of Dry Chemicals. Dry C. P. chemicals, such as dextrose, sodium chloride, and sodium citrate, are weighed upon large clean filter papers or in dry beakers on a bench model beam balance, after the tare of the paper or beaker has been counterbalanced. The scales are kept clean and covered to prevent accumulation of dust. The chemicals are kept in the large drums in which they are shipped. They should be kept tightly covered to prevent entrance of moisture and dirt. It is convenient to store the drums on small platforms with casters, so that they may be pushed under the table when not in use, and so that they can be easily moved in cleaning the floor.

Mixing. If a milk can is used as a mixing tank, it should previously be cleaned, dried, and stored with a dust-proof cover. The tank is weighed dry on the platform scale on the floor, and the tare is counterbalanced. The can is then washed with a liberal amount of pyrogen-free distilled water, which is discarded. The rinsed can is placed on the scale and pyrogen-free distilled water is run into it until the proper weight is registered. The filled can is lifted onto a small platform with casters, rolled under the shaft of the motor mixer, and anchored securely. The mixing shaft is lowered into the water and the motor is turned on cautiously so that splashing does not occur. When the water is in motion, the dry chemicals are poured into the water, small amounts at a time so that they do not cake on the bottom of the can. When mixing is complete, the shaft of the mixer is raised out of the solution and permitted to drain for a short time. The can is then wheeled away and the mixer is cleaned, dried, and covered with a dust-proof shield.

Filtration. The filled can is rolled near the bench model filter. The filter has previously been washed with pyrogen-free distilled water and dried. New filter pads are inserted and a liberal amount of pyrogen-free water is pumped through the filter and discarded. The end of the intake hose of the filter is placed in the bottom of the tank of solution and the contents pumped through the filter

into another clean can. After use, the filter pads are discarded and the machine is cleaned and dried.

Bottling. The tank of solution is rolled to the bottling machine and the containers are filled to a predetermined level. If the closures are plastic caps, they are placed loosely on the tops of the bottles to permit free access and egress of air. If the flasks are to be evacuated rubber closures are placed tightly.

Evacuating Bottles. If vacuum bottles are desired for the collection of blood, each closure is pierced with a 17 gauge needle attached by a piece of thick-walled rubber tubing to a vacuum pump and a mercury manometer. When a vacuum of about 54 cm. of mercury is drawn the needle is withdrawn.

Sterilization. The filled containers are placed on a cart which is wheeled into the autoclave. When no evacuation of the bottle has been effected, the caps should fit loosely and several spare wrapped closures should be placed in the autoclave to replace any which may be blown off later or displaced by solutions boiling over. The evacuated flasks are autoclaved after the vacuum has been drawn. The autoclave is closed and the temperature is gradually run up to 121° C. (250° F.) for twenty minutes. The pressure is then permitted to fall gradually to that of the atmosphere. If the pressure falls too fast, the fluid in the flasks will boil over. The hot solution flasks are then removed from the autoclave and caps which have blown are replaced aseptically.

If the flasks are not evacuated, the operator tightens each screw cap while the solution is hot, protecting his hands by canvas or asbestos gloves. When the flasks cool, a vacuum is formed.

Labeling. Flat bottles may be laid on the side after they have been tightly stoppered. This maneuver will reveal any leaks which may be present around the closure as the bubbles of air will be seen rising to the surface of the liquid. Round bottles must be in the vertical position for labeling. Gummed labels may be pulled from a dispensing machine which wets them and cuts them off. They are placed on the bottles by hand. The date of processing is then placed on the label with a rubber stamp.

Testing for Leaks. Bottles which contain a vacuum obviously have not leaked to any extent. The vacuum can be tested for by having the personnel hold the containers horizontally and striking the bottom of the container with the palm of the hand. If the space in the bottle is evacuated the fluid will produce a sharp click as it hits the opposite end of the container.

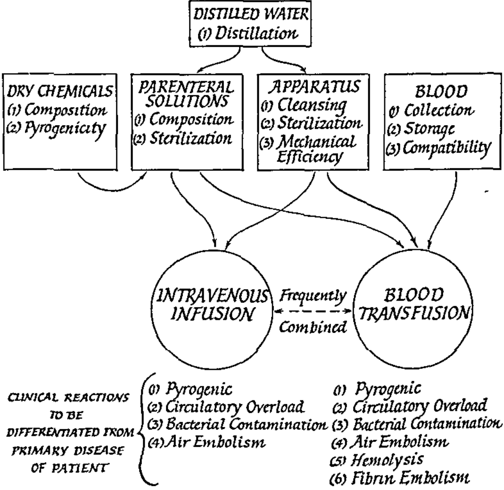
Opening Bottles. When the containers have been closed with screw caps while the fluid is hot, the resulting vacuum makes manual opening difficult. Persons who are to open the containers should be equipped with devices for turning the caps.

SOURCES OF PYROGEN CONTAMINATION

The flow chart shows graphically that pyrogens may be introduced at many points in the production of blood preservative mixtures and solutions for parenteral therapy. The problem of searching for the source of pyrogen contamination in blood transfusions is inextricably linked with the distillation of water, the manufacture of parenteral solutions, the preparation of apparatus for transfusion

FLOW CHART FOR PROCESSING OF TRANSFUSIONS AND INFUSIONS

(Pyrogens may be introduced any place in the line.)



and infusion, and the operation of a blood bank. Frequently, also, the same patient receives both blood transfusion and crystalloid infusions consecutively. It is therefore logical and necessary that the transfusionist should have technical direction of all procedures which may contribute pyrogens to the blood, since in most cases he is the only physician who is qualified and able to diagnose pyrogenic reactions in patients and has a detailed interest in the subject.

EPIDEMIOLOGIC METHOD OF PYROGEN DETECTION

In searching for the source of pyrogen contamination the technical director must analyze a very complicated situation, as depicted in the flow diagram. The use of biologic tests for pyrogens has been of little aid except when the parenteral solutions have been indicated by other evidence. An epidemiologic approach has been evolved which has proved successful. It depends on the following principles: (1) Accurate reporting of the occurrence or absence of chills and fever associated with all intravenous infusions and blood transfusions. (2) The use of the date of preparation as a lot number for each flask of parenteral fluid and blood preservative solution. (3) The date of preparation of each set of infusion and transfusion apparatus is used as a lot number. (4) A report is made of the unit of the hospital in which the patient received the injection, whether pyrogenic or not. (5) An effort is made to insure that the solution and the infusion set concerned in the injection of a patient are processed on different days. (6) The distilled water for the washing of apparatus and the processing of solutions is derived from the same still. The chemicals for the crystalloid solutions and for the blood preservative mixtures have the same source as far as possible. (7) The rubber tubing and accessories are used interchangeably for transfusion and infusion apparatus, as much as possible.

Method of Reporting. A small printed shipping tag, tied to the tubing, is packed in each transfusion and infusion set and sterilized in the package. The tag is stamped with the date of sterilization of the set. The soiled set cannot be returned to the Central Preparation Department until all the data have been recorded on the tag with a *lead pencil* (to prevent effacement when wet). The nurse who discontinues the transfusion or infusion at the patient's bedside is made responsible for recording the data on the tag. The information required is: (1) Name of patient, (2) Date of administration of infusion or transfusion, (3) Date of sterilization of the set (this has been filled in during the preparation), (4) Dates of preparation of all flasks of fluid used with the injection and the nature of solution employed. The date of preparation of the blood preservative solutions is recorded in the blood bank. (5) A check mark, if blood was injected with the set. (6) A written "Yes" or "No" as to whether chills and fever accompanied the injection. (7) Name of the nurse recording the data. (8) The unit of the hospital in which the injection was given. (9) Notations on mechanical imperfections in the apparatus.

The investigation of blood transfusion reactions is made independently by examination of the recipient and testing of the blood involved by personnel from the blood transfusion service.

Analysis of Data. The data on all infusions and transfusions are plotted daily on a graph by the intern on the blood transfusion service. This is considered part of his education on transfusion reactions. Separate graphs are made for blood transfusions and infusions of crystalloid solutions.

The graph for crystalloid infusions contains a black dot for each infusion without reaction and a red point for one with reaction. The abscissas are laid off for the dates of preparation of fluids and the ordinates are marked with the dates of preparation of the infusion sets. The same plan is used for graphing blood transfusions. Subsidiary graphs may be constructed for dextrose infusions and saline injections.

A preponderance of reactions on the date of sterilization of a lot of fluids or that of a number of infusion sets may usually be seen by inspection of the graphs. If no preponderance is noted by that method of analysis, the incidence of reactions occurring in different units of the hospital may be obtained from the report tags. When one unit shows a particularly high incidence of reactions with fluids and sets of lots, which are not causing difficulty in other areas, the procedures in that unit are investigated by interviewing nursing and medical personnel concerned. Usually it will be discovered that apparatus prepared locally has been attached to the sets or else pyrogenic solutions of vitamins, amino acids, penicillin, streptomycin, or other products are being added to the crystalloid solutions.

If the incidence of reactions in blood transfusions is disproportionately high, as compared with crystalloid infusions, the problem is narrowed considerably. Distilled water from the same source is employed in the preparation of the transfusion and infusion sets and in the manufacture of solutions. Dextrose and saline are common to both crystalloid infusions and blood preservative solutions. There is no control injection intravenously of sodium citrate or citric acid not associated with blood. Filters are used in blood transfusion but not with the injection of crystalloid solutions. The possibility of pyrogens in the crystalline chemicals or the filters must then be considered.

A spotty distribution of reactions, without apparent correlation with sets or solutions, strongly suggests imperfect preparation of apparatus nevertheless. After a clue has been obtained by the epidemiologic analysis, the procedure which has been indicted is investigated in detail, utilizing a thorough knowledge of the production of pyrogens. Usually the errors will be discovered in a short time. The biologic test for pyrogens can be made on the water coming from the stills, but if the offending substances are in the infusion equipment, even tests of the solutions remaining in the

container after a reaction has been produced are negative because the pyrogens have been washed into the veins. If the infusion apparatus is known to be pyrogenic but the solutions are negative, the equipment can be washed out by running through it and discarding the first 200 ml. of fluid before the injection is started.

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(Note: All illustrations will be found under the heading *Diagrams and Drawings*.)